REMARKS

A Request for filing an RCE and a check for the fees for filing an RCE and for a two-month extension of time accompany this paper. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 50-52 and 74-113 are pending. Claims 55-57 are cancelled herein without prejudice or disclaimer. Applicant reserves the right to file divisional and continuation applications to such subject matter. Claim 50 is amended to depend from new claim 92. Basis for this amendment can be found, for example, in the specification at page at page 48, line 11 to page 52, line 10, page 54 line 1 to page 55 line 3 and page 12, line 25 to page 13, line 7 and in the claims as originally filed. Claim 51 is amended herein to correct an inadvertent obvious typographical error made in the renumbering of the claims in the Response and Amendment of November 1, 2002. Claims 51 is amended to properly depend from claim 50. Claim 52 is amended to more clearly describe the claimed subject matter. Basis for this amendment can be found for example, at page 48, line 11 to page 52, line 10 and at page 54 line 1 to page 55 line 3. Claims 74-113 are added. The amendment and new claims find basis in the specification as originally filed. For example, claims 74-113 find basis in the specification, for example, at page 48, line 11 to page 52, line 10, page 54 line 1 to page 55 line 3 and page 12, line 25 to page 13, line 7. The amendments and new claims also find basis in the claims as originally filed.

Applicant makes reference throughout this paper to the Response and Amendment, mailed April 22, 2004, hereinafter referred to as the "Response." The text of the "Response" is incorporated herein by reference. A Declaration under 37 C.F.R. § 1.132 of Dr. Steven Fabijanski accompanies this response (referred to as Declaration 3 herein).

Rejection of claims 50-52 under 35 U.S.C. §112, second paragraph

Claims 50-52 are rejected under 35 U.S.C. §112, second paragraph, as indefinite because the phrase "satellite artificial chromosome" allegedly is unclear. Responsive to Applicants arguments submitted in the "Response" of April 22, 2004, the Office Action urges that these arguments are unpersuasive because the Applicant has provided no structural information regarding the chromosomes such as, for example, the order of centromeres, telomeres, origin of replication and filler heterochromatin. It is alleged that the terms used to

define a satellite artificial chromosome are unclear, including the differences between the terms " a fully functional stable chromosome, and a "functional, stable artificial chromosome." It is alleged that a recitation of the properties of a satellite artificial chromosome does not recite essential elements. It also is alleged that since a "functional stable artificial chromosome" includes a centromere, telomeres and at least one origin of replication, it is unclear how the satellite artificial chromosome can "have no genetic information." It is further alleged that the metes and bounds of a satellite artificial chromosome are not apparent.

Responsive to the argument set forth in the "Response" that the term "satellite artificial chromosome" describes a heretofore unknown product, like other pioneering inventions such as the telephone and automobile, the Examiner states that unlike the patent applications for the telephone and automobile, no drawings, figures or working models of satellite artificial chromosomes are provided in the instant application. In response to Applicant's submission that the term "satellite artificial chromosome" is presumptively definite because satellite artificial chromosomes are claimed in a parent applications that issued as U.S. patents, the Examiner asserts that each case is different and evaluated on its own merits.

This rejection is respectfully traversed.

Summary of Arguments below

Applicant respectfully submits that the term "satellite artificial chromosome" (also referred to herein as SATAC) defines the metes and bounds of the term in accord with the requirements of 35 U.S.C. §112, second paragraph. The arguments below address three points that contravene the Examiner's assertions:

A. "Satellite artificial chromosome" is sufficiently defined and described in the specification so that the metes and bounds of the term are clear. The specification provides the term "satellite artificial chromosome" to describe a heretofore unknown type of chromosome. A "satellite artificial chromosome" is a chromosome that is contains more heterochromatin than euchromatin. As described in great detail in the specification, it is composed of repeating units of satellite DNA. It contains more heterochromatin than euchromatin. Each of the elements of this definition are fully described and exemplified in the instant application. In fact, a large portion of the application is directed to describing SATACs, their preparation and uses thereof.

B. The Examiner's assertion regarding the lack of working examples and drawings in comparison of Applicant's pioneering discovery, the SATAC, to other pioneering inventions such as the telephone and automobile is without merit. To the contrary, as discussed below, SATACS are described in detail in the specification, depicted in figures and exemplified in working examples.

C. The Examiner's assertion that claims to satellite artificial chromosomes and related methods in the two issued patents, U.S. Patent Nos. 6,077,697 and 6,025,155, have no bearing on the definiteness of this term in the instant case is incorrect. While unrelated cases may be treated on each case's own merit, the instant application and the aforementioned two issued patents are based upon the same application(s) and find descriptive basis in the *same* application. The instant claims, including the elements of a SATAC as used in the claims, find basis in U.S. application serial nos. 08/695,191, 08/682,080 and 08/629,822, to which the instant application claims priority. These applications issued as U.S. Patent Nos. 6,025,155 and 6,077,697, respectively.

Furthermore, the instant application and the issued patents claim priority to the earlier filed application, U.S. application serial no. 08/629,822, filed April 10. 1996. This application describes SATACs, elements of SATACs and methods of making SATACs. The issued claims of U.S. Patent Nos. 6,025,155 and 6,077,697 to satellite artificial chromosomes, cells containing satellite artificial chromosomes and methods of preparing satellite artificial chromosomes, find basis in U.S. application serial no. 08/629,822. The instant application also claims priority to U.S. application serial no. 08/629,822, and the instant claims find basis in this application. Accordingly, the instant claims, including the element "satellite artificial chromosome" in the claims, are based on the same disclosure as claims in the issued patents. In particular, the description and claims upon which the instant claims are based is found in U.S. application serial no. 08/629,822, the same priority document that provides basis for the claims in the issued parent patents. The issued parent patents of this application, U.S. Patent Nos. 6,025,155 and 6,077,697, contain claims to satellite artificial chromosomes *per se*, cells containing satellite artificial chromosomes and methods of preparing satellite artificial chromosomes. Thus, recitation of "satellite artificial chromosome" in this application is presumptively definite. The Patent Office cannot

denigrate the validity of issued patents and must give full faith and credit to its own findings. MPEP §1701.

DETAILED ANALYSIS

A. "Satellite artificial chromosome" is sufficiently defined and described in the specification so that the metes and bounds of the term are clear

It is respectfully submitted that recitation of the term "satellite artificial chromosome" is definite. Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. Rosemount Inc. v. Beckman Instruments, Inc., 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), Caterpillar Tractor Co. v. Berco, S.P.A., 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). When one skilled in the art would understand all of the language in the claims when read in light of the specification, a claim is not indefinite. An applicant is entitled to be its own lexicographer [see, *e.g.*, MPEP 2111.01 "Applicant may be his or her own lexicographer as long as the meaning assigned to the term is not repugnant to the term's well known usage and employ terms within the claims that are clear from a reading of the specification. In re Hill, 73 USPQ 482 (CCPA 1947)."].

Satellite Artificial Chromosome

As explained herein and in the "Response" in great detail, "satellite artificial chromosome" refers to a chromosome that contains more heterochromatin than euchromatin, that is composed of repeating units of short satellite DNA, and that contains more heterochromatin than euchromatin (see, *e.g.*, page 7, lines 15-20, page 19, lines 4-6 and page 94, lines 3-21). The specification also describes that a satellite artificial chromosome can contain interspersed heterologous DNA in addition to heterochromatic nucleic acid (page 19, lines 4-6). The application is directed to teaching how to make and use satellite artificial chromosomes, and includes definitional language as well as diagrammatic representations depicting the structure of these chromosomes and working examples, describing how to make them and how to identify them, such as by their characteristic banding patterns upon staining.

35 U.S.C. §112, second paragraph, requires only reasonable precision in delineating the bounds of the claimed invention. Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject

matter permits. Shatterproof Glass Corp. v. Libby-Owens Ford Col., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

Each of the terms used to describe a satellite artificial chromosome is a term known in the art

Each of the terms used in the application to describe a satellite artificial chromosome; "chromosome," "heterochromatin," "satellite DNA, "euchromatin" and "heterologous DNA" are all well-known terms in the field of cell and molecular biology (see, for example, Klug, W.S. and Cummings, M.R. (1983) Concepts of Genetics, pp. 201-204, provided herewith). The specification further provides extensive descriptions of each of the terms in the definition of a satellite artificial chromosome and describes exemplary embodiments of chromosomes that fall within the definition of a satellite artificial chromosome.

Heterochromatin and Euchromatin

For example, the specification describes heterochromatin as chromatin that is unusually condensed and thought to be transcriptionally inactive (at page 17, lines 27-31). Further, the specification describes that chromosomes containing heterochromatin can be distinguished from those containing euchromatin by staining patterns as well as by differences in their gene content (see, e.g., page 17, lines 27 to page 18, line 5).

Heterologous DNA

As noted the "satellite artificial chromosomes" can contain heterologous DNA. At page 21, lines 29 to page 22, line 3, the specification defines heterologous DNA as DNA that does not occur naturally as part of the genome or that is in a new locus in the genome or that would otherwise be considered "heterologous" by one of skill in the art. Page 22, lines 3-13 of the specification provides examples of heterologous DNA that can form part of the satellite artificial chromosome.

Fully functional stable chromosome

Moreover, the very terms such as "a fully functional stable chromosome" and a "functional, stable artificial chromosome," that the Examiner alleges are not addressed, are defined and described in the application. For instance, the specification defines an artificial chromosome as a piece of DNA that can replicate and segregate alongside endogenous chromosomes as well as accommodate heterologous DNA (see, for example, page 17, lines 8-9). Thus, the application clearly distinguishes an artificial chromosome as one that is not found as an endogenous chromosome in the cell. "Functional" is set forth, for example, in

the definition of artificial chromosomes and includes the ability of the artificial chromosome to replicate and segregate alongside endogenous chromosomes. The application further discusses stability. Stability can be measured by the percentage of cells that retain the chromosome in the presence of a selective agent, generally 85% or greater. Additional stable chromosomes refer to chromosomes that do not have intrachromosomal or interchromosomal rearrangements when maintained in cell culture (for example, at page 17, lines 10-16). Additionally, as noted above, terms such as "artificial chromosome," "chromosome" and the stability and function of such chromosomes are well-known terms and concepts in the art.

No genetic information

The specification describes satellite artificial chromosomes as containing more heterochromatic DNA than euchromatic DNA. As explained in the application, SATACs contain more heterochromatic DNA than euchromatic DNA. As known to those of skill in the art and as described in the application, heterochromatic DNA is generally non-coding DNA. Therefore, to the extent a SATAC is composed of heterochromatic DNA it does not encode genetic information.

Hence, as described in great detail in the previous "Response," each of the elements of a satellite artificial chromosome are clearly defined in the specification so that the metes and bounds of the term "satellite artificial chromosome," in light of the specification and as understood by those of skill in the art, are clear. The Examiner is additionally referred to pages 4-9 of the "Response" for a detailed discussion.

The linear order of the elements of the satellite artificial chromosome are not necessary for the definition of a satellite artificial chromosome

The Office Action alleges that the Applicant has provided no structural information because a linear order of a centromere, telomeres, origin of replication and heterochromatin is not provided. Applicant respectfully disagrees.

"To satisfy this requirement, the claim, read in light of the specification, must apprise those skilled in the art of the scope of the claim." SmithKline Beecham Corp. v. Apotex Corp, 365 F.3d 1306, 1314 (Fed. Cir. 2004); See also Miles Labs., Inc. v. Shandon, Inc., 997 F.2d 870, 875 (Fed.Cir.1993). The linear order of elements is not necessary to describe a satellite artificial chromosome and apprise one of skill in the art of the claimed subject matter. As explained above, satellite artificial chromosomes can be distinguished from endogenous chromosomes based on their content of heterochromatin as compared to

euchromatin. The specification also describes that satellite artificial chromosomes contain the requisite elements of endogenous chromosomes including centromere, telomeres and at least one origin of replication, for stable maintenance and replication. Such features are sufficient to for one of skill in the art to recognize what Applicant has claimed. No more is required.

B. Contrary to the Examiner's assertion, detailed drawings, figures and working examples of satellite artificial chromosomes are provided in the specification

It is respectfully submitted that the Examiner's assertion that the satellite artificial chromosome, unlike other pioneering discoveries like the automobile and the telephone, is not supported by sufficient working examples and drawings in the specification, is without merit. As noted above, an applicant can be its own lexicographer. Here, Applicant has employed "satellite artificial chromosome" to refer to the chromosomes described in the application that contain more heterochromatin than euchromatin. In the "Response," Applicant analogized:

> First, it is noted that Applicant has discovered and provides something so new that no name existed to describe the chromosomes. Before someone had built the first automobile or telephone, the terms "automobile" or "telephone" would not have been clear. Just as the inventor of each of these items had to name them, Applicant coined its own name for its new vectors, and called them "satellite artificial chromosomes."

This analogy was employed to emphasize the fact that one cannot use a known name for something when one has invented something truly new; one cannot use a known name for something that heretofore did not exist. In response to the above arguments, the Examiner responds that because Applicant has not provided drawings, figures and working models of satellite artificial chromosomes such as provided with the first telephone and automobile applications, Applicant has not fully described the claimed subject matter. It is respectfully submitted that this is not correct. Most of the application is devoted to a description of these chromosomes, including how to make and use them, and their structure.

Drawings and Figures

Applicant respectfully points out that numerous drawings and figures of satellite artificial chromosomes are provided in the specification. Further, the drawings and figures were discussed in the "Response." For example, the specification depicts the structures of satellite artificial chromosomes schematically in Figures 2 and 3. In particular, the formation

of a megachromosome, which is a satellite artificial chromosome, is shown in Figures 2D, 2E and 2F and the structure of an exemplary megachromosome is depicted in Figures 2F and 3. These drawings were based on detailed studies of satellite artificial chromosomes to observe the chromosome architecture within a satellite artificial chromosome (see, for example, pages 93-110), including *in situ* hybridization with satellite DNA as a probe for heterochromatic DNA and hybridization with euchromatic probes (for example, pages 94-95), Hoechst staining (for example, page 95) and FPG staining (for example, page 96). In addition, pulse field gel electrophoresis and southern hybridization were used to map satellite artificial chromosomes (for example, page 96). Cloning and sequencing of regions of satellite artificial chromosomes were employed to confirm the structure of the chromosome (for example, pages 97-98). Additionally, such satellite artificial chromosomes were observed directly by scanning electron microscopy (for example, page 97).

The specification provides detailed guidance for preparing a satellite artificial chromosome, and exemplifies preparation of several different satellite artificial chromosomes and cells containing satellite artificial chromosomes. For example, at pages 33-39, the specification contains a section entitled "Preparation of SATACs." The section includes detailed descriptions of methods of making satellite artificial chromosomes, and descriptions of satellite artificial chromosomes and their structure. The section includes a detailed description of Figures 2D, 2E, 2F and Figure 3 that depict structures of satellite artificial chromosomes and the formation of satellite artificial chromosomes. In addition, this section of the specification includes reference to two cells lines, G3D5 and H1D3, each containing a megachromosome (an example of a satellite artificial chromosomes) and each deposited with the ECACC (see, for example pages 36-39).

Working Examples

The specification also provides working examples that detail how to produce satellite artificial chromosomes and a details characterization of satellite artificial chromosomes; provides satellite artificial chromosomes, and cells with satellite artificial chromosomes, including deposited cell lines. Examples 4 and 5 describe the formation of satellite artificial chromosomes and formation of exemplary intermediate structures. Example 6 describes three exemplary cell lines, G3D5, H1D3 and mMC21, containing satellite artificial chromosomes. The example provides description of the satellite artificial chromosomes, including characterization of their dominating heterochromatic structure. Example 6 also

details the use of hybridization, Hoechst staining and scanning electron microscopy to characterize satellite artificial chromosomes and cells with satellite artificial chromosomes. The example describes in detail the results of such assays and the features of the satellite artificial chromosomes identified by such assays. Detailed references to the figures, such as Figure 2 describing the structure of satellite artificial chromosomes, also are included in Example 6. In addition, the example describes satellite artificial chromosomes that contain heterologous DNA, for example a selectable marker and/or a reporter gene. Further, Example 8, at page 113 of the specification, describes in great detail the *in vivo* replication of a megachromosome. Example 10, at page 124 of the specification, describes in great detail methods for the isolation of satellite artificial chromosomes from endogenous chromosomes based upon the atypical base content and/or size of the satellite artificial chromosome. Example 12, at page 140 of the specification, describes in great detail the preparation of vectors and plasmids, such as the λCF-7 and the λCF-7-DTA vectors and the pMCT-RUC and the pLNCX-ILRUC plasmids, for the targeted integration of heterologous DNA into artificial chromosomes. Example 13, at page 165 of the specification, describes methods for the microinjection of artificial chromosomes into eukaryotic cells, and detection of expression of the encoded heterologous DNA (β-Gal). In addition, the specification provides working examples that demonstrate the production and delivery of satellite artificial chromosomes in other cell types. For example, Example 9 demonstrates generation of SATACs from a different chromosome (chromosome 1). Example 10 describes the production of microcells containing satellite artificial chromosomes and transfer of satellite artificial chromosomes from microcells into a second cell type. Example 11 describes the transfer of artificial chromosomes from one species into another, in particular the transfer of a mammalian artificial chromosome into insect cells. Hence, the specification provides detailed description of satellite artificial chromosomes and cells containing satellite artificial chromosomes, including detailed figures and working examples that describe and exemplify satellite artificial chromosomes.

Additionally, for exemplification, Applicant has deposited cells lines containing satellite artificial chromosomes and cell lines containing intermediates in the methods of making satellite artificial chromosomes at the European Collection of Animal Cell Culture (ECACC) (see, for example page 74, line 22 to page 75, line 7). Such cell lines and the deposits are described in the parent U.S. application serial no. 08/682,080. During the

prosecution of parent U.S. application serial no. 08/682,080, Applicant stated that all restrictions upon availability of the deposited material would be removed upon granting of a patent that included claims to the deposited material. Thus, upon issuance of U.S. Patent No. 6,077,697 with claims to such cell lines, the cell lines are available without restriction as "working models" (see Amendment filed October 1, 1997 in the prosecution of parent U.S. application serial no. 08/682,080).

C. The term "Satellite artificial chromosome" is presumptively definite; in issuing patents, based on the same application, with claims to satellite artificial chromosomes, the Office has concluded that the metes and bounds are clear

The instant application is a continuation-in part of U.S. application serial nos. 08/695,191, 08/682,080 and 08/629,822. Two U.S. patents have issued based on these applications: U.S. Patent Nos. 6,077,697 and 6,025,155. U.S. Patent No. 6,077,697 is based upon U.S. application serial nos. 08/682,080 and 08/629,822; U.S. Patent No. 6,025,155, is based upon U.S. application serial nos. 08/695,191and 08/629,822. Thus, the instant application and the issued parent patents are all continuations-in-part of U.S. application serial no. 08/629,822. Moreover, the claims of the instant application and the claims of the issued parent patents find basis in the disclosure of U.S. application serial no. 08/629,822, filed April 10, 1996. Hence, the pending claims of the instant application are based on the same disclosure in which the issued claims find basis.

The issued parent patents, U.S. Patent Nos. 6,025,155 and 6,077,697, contain claims to satellite artificial chromosomes *per se*, cells containing satellite artificial chromosomes and methods of preparing satellite artificial chromosomes. patents include claims to satellite artificial chromosomes, cells containing satellite artificial chromosomes and methods of making and using such chromosomes and cells. For example, U.S. Patent No. 6,077,697 includes the following claims:

6. A cell containing a satellite artificial chromosome.
8. An isolated substantially pure satellite artificial chromosome.

U.S. Patent No. 6,025,155 includes claims such as:

22. A method for producing a gene product or products comprising: introducing satellite artificial chromosomes comprising DNA encoding the gene or gene products into cells; and culturing the cells under conditions whereby the gene product or products are expressed.

As noted above, the issued patents, U.S. Patent Nos. 6,077,697 and 6,025,155 claim priority to U.S. applications serial nos. 08/695,191, 08/682,080 and 08/629,822. Moreover,

the issued claims find basis in U.S. applications serial no. 08/629,822. The claims of the instant application also find basis in U.S. applications serial no. 08/629,822, to which the instant application claims priority. Additionally, the disclosures of U.S. application serial no. 08/629,822 (and U.S. application serial nos. 08/695,191, 08/682,080) are incorporated by reference in their entirety into the instant application. ***Hence, the claims of the instant application find basis in the same disclosure as the issued claims.***

Therefore, the Patent Office having issued claims to satellite artificial chromosomes and cells containing satellite artificial chromosomes based on the disclosures of U.S. application serial no. 08/629,822 in U.S. Patent Nos. 6,077,697 and 6,025,155, has determined that the phrase is definite and can not now assert that the term "satellite artificial chromosomes" is indefinite. As set forth in 35 U.S.C. §282, an issued patent is presumptively valid and meets the requirements of definiteness. Furthermore the MPEP states that the Examiner must give full faith and credit to determinations of the Patent Office and cannot denigrate the validity of an issued patent. MPEP §1701.

The Examiner alleges that each case is different and evaluated on its own merit. ***While this statement may be true for unrelated applications, Applicant respectfully submits that the issued patents, U.S. Patent Nos. 6,077,697 and 6,025,155, and the instant application are part of the same patent chain and rely on identical disclosure, U.S. application serial no. 08/629,822 and claim priority to the same application.*** As noted above, the issued claims in U.S. Patent Nos. 6,077,697 and 6,025,155, that include claims directed to satellite artificial chromosomes, cells containing satellite artificial chromosomes and methods for producing gene products from satellite artificial chromosomes, find basis in U.S. application serial no. 08/629,822. These issued claims are presumptively definite. 35 U.S.C. §282. The instantly rejected claims to methods of producing a cell containing heterologous DNA by introducing a SATAC, also find basis in U.S. application serial no. 08/629,822. Hence, the claims in the instant application find basis in the identical disclosure as issued U.S. Patent Nos. 6,077,697 and 6,025,155, which include claims that recite "satellite artificial chromosome." Therefore, the term "satellite artificial chromosome" is presumptively definite.

In summary, in light of the remarks above, demonstrating the extensive description and definition of the term "satellite artificial chromosome" present in the disclosure, in

addition to the presumptive validity of the term based on issued claims in parent patents, Applicant respectfully requests that this rejection be withdrawn.

II. The Rejection under 35 U.S.C. §112, first paragraph: Written Description

The Final Office Action maintains the rejection of claims 50-52 under 35 U.S.C. §112, first paragraph, for reasons of record. It is alleged that the specification does not describe the subject matter in such a way as to convey to one skilled in the relevant art that the inventor(s) had possession of the claimed subject matter at the time the application was filed. In particular, it is alleged that the identifying characteristics set forth in the specification for a satellite artificial chromosome or a plant artificial chromosome are insufficient to demonstrate possession of satellite artificial chromosomes and plant satellite artificial chromosomes by the Applicant. Further, it is alleged that insufficient relevant identifying characteristics are set forth to "predictably" determine the structure and function of a satellite artificial chromosome in order to use the satellite artificial chromosome as the starting material for producing a cell containing a satellite artificial chromosome or a plant satellite artificial chromosome and then identifying the cell containing the satellite artificial chromosome or plant satellite artificial chromosome.

In response to Applicant's arguments in the "Response," the Examiner alleges the following additional points: Applicant allegedly fails to describe an alleged essential component of artificial chromosomes; Applicant allegedly has not distinguished artificial chromosomes from wild-type chromosomes; the deposit of cell lines and the depiction of satellite artificial chromosome the Figures of the application are allegedly insufficient to describe satellite artificial chromosomes; and Applicant can not rely on the disclosures of parent patents.

This rejection is respectfully traversed.

Summary of Arguments

Applicant respectfully submits that the application provides sufficient written description of satellite artificial chromosomes to demonstrate Applicant's possession of satellite artificial chromosomes at the time of filing and as of the earliest priority date of the instant application. The arguments summarized here and addressed in further detail below, address the points of this rejection.

A. The specification provides sufficient identifying characteristics of satellite artificial chromosomes, including plant satellite artificial chromosomes, to evidence Applicant's possession of the claimed subject matter as of the filing date of the instant application.

In the "Response" filed in connection with the previous Office Action of September 30, 2003, Applicant cited numerous descriptions in the specification, demonstrating that Applicant had possession of the claimed subject matter as of its earliest priority date and at the time of filing. As further described below, the "Response" pointed to descriptions in the specification, working examples and figures depicting the claimed subject matter, all evidencing Applicant's possession of the claimed subject matter at the time of filing. The Examiner also is referred to pages 15-19 of the "Response."

Although the Examiner alleges that Applicant's description lacks an essential feature, namely proteins associated with the chromosomes, such statement is inapt. Applicant is not required to describe what is known in the art. Chromosomes *per se*, including associated proteins, were well known and characterized. Applicant has described the unique identifying features of satellite artificial chromosomes that distinguish them from other chromosomes known in the art. Applicant has described a wide variety of cell types containing satellite artificial chromosomes, including working models of stable and functional satellite artificial chromosomes. Furthermore, the Examiner has provided no basis to doubt that Applicant possessed satellite artificial chromosomes and cells containing satellite artificial chromosomes at the time filing of the application and as of the earliest priority date. Thus, applicant has provided sufficient description to satisfy the written description requirement.

B. Satellite artificial chromosomes are sufficiently described in the instant application to "predictably" determine the structure and function of a satellite artificial chromosome in order to use the satellite artificial chromosome as a starting material for producing and identifying a cell that contains a satellite artificial chromosome, including a plant cell that contains a plant satellite artificial chromosome.

Although Applicant maintains that the issue of predictability goes to a determination of enablement and is not pertinent to a consideration of written description, this point also was addressed with respect to the written description rejection in the "Response." As discussed in detail in the "Response," and summarized herein below, the instant application describes satellite artificial chromosomes and cells containing satellite artificial chromosomes in extensive detail so that satellite artificial chromosomes and cells containing satellite

artificial chromosomes can be reproducibly and "predictably" produced and identified by one of skill in the art. The application describes methods for generating satellite artificial chromosomes of any plant and animal species that are applicable to any plant and animal cell type. The application provides methods for generating satellite artificial chromosomes , for example, by introducing heterologous DNA with a selectable marker and/or sequences targeted to the pericentric region of a chromosome into a cell, growing cells under selective condition and selecting cells that have incorporated the DNA and contain a satellite artificial chromosome. The application provides working examples of these methods. Further, although the working examples are exemplified in mammalian cells, the application explains that these methods are applicable to any plant and animal cell type and any plant and animal species of satellite artificial chromosomes. The application provides descriptions of the elements of satellite artificial chromosomes and distinguishing features, such as a the presence of more heterochromatin than euchromatic. Further, the application provides methods for identifying satellite artificial chromosomes that are applicable to all cell types.

Again, in view of the extensive descriptions provided, Applicant respectfully submits that the Examiner has provided no basis to doubt that Applicant possessed satellite artificial chromosomes and cells containing satellite artificial chromosomes at the time filing of the application and as of the earliest priority date.

C. Applicant had possession of the claimed subject matter as of the filing date of the earliest priority document.

In the "Response," Applicant noted that the instant application is part of the ***same patent family***, related as a continuation-in-part, to the parent applications U.S. application serial nos. 08/695,191, 08/682,080 and 08/629,822, from which two U.S. patents have issued, U.S. Patents Nos. 6,077,697 and 6,025,155. These issued patents, which presumptively are valid, have issued claims to satellite artificial chromosomes, cells containing satellite artificial chromosomes and methods for producing gene products from satellite artificial chromosomes. The disclosure relied on by the Examiner in alleging that the instant application fails to describe a satellite artificial chromosome, incorporates each of the parent disclosures by reference and provides descriptions of a satellite artificial chromosomes that are substantially ***identical*** to the disclosures of the parent applications upon which the issued patents are based. Therefore, the specification provides written description therefor.

The Examiner alleges that "each case is different." As discussed in detail below and above, this statement is inapt as applied to the instant application. ***The issued patents and the instant application are related as continuation-in-part applications of a common parent applications, find basis in the same description, and are thus the same "case."*** The subject matter that forms basis for the instant claims is identical among all parent applications, U.S. application serial nos. 08/695,191, 08/682,080 and 08/629,822. Moreover, the subject matter that forms basis for the instant claims and the claims of U.S. Patents Nos. 6,077,697 and 6,025,155 finds basis in U.S. application serial no. 08/629,822, to which the instant application and the parent patent applications claim priority. Therefore, Applicant has demonstrated possession of satellite artificial chromosomes as of this application's earliest effective filing date.

DETAILED ANALYSIS

A. ***The specification provides sufficient identifying characteristics of satellite artificial chromosomes, including plant satellite artificial chromosomes, to evidence Applicant's possession of the claimed subject matter as of the filing date.***

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir.1989). The written description for a claimed genus can be satisfied by disclosure of identifying characteristics, including structural and physical characteristics, functional characteristics coupled with known or disclosed correlation with structural characteristics or a combination of such factors sufficient to demonstrate that the applicant was in possession of the claimed subject matter. MPEP § 2163; *see* University of California v. Eli Lilly, 119 F. 3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

The instant application provides detailed identifying characteristics of satellite artificial chromosomes including detailed descriptions and drawings evidencing Applicant's possession of the claimed subject matter. For example, the section of the specification entitled "Preparation of SATACs" at page 33, line 19 to page 36, line 23, provides detailed descriptions of satellite artificial chromosomes and their characteristic structure. Cell lines containing satellite artificial chromosomes are described at page 36, line 24 to page 39, line 24. Additionally, Example 6, pages 92-93, describes production of an exemplary satellite artificial chromosome. Example 8 describes labeling and *in situ* studies of a

megachromosome, an exemplary satellite artificial chromosome. The example characterizes replicating regions and features of the chromosomes. Example 9 describes the production of large scale amplification of pericentric regions in cells and analysis of the chromosomal products.. Example 10 describes the purification of satellite artificial chromosomes using identifying characteristics such as atypical base content and size. The example describes the use of dyes such as Hoechst and chromomycin to stain and sort the chromosomes. Each of these examples provide detailed description and characterization of the satellite artificial chromosome structure. These detailed descriptions of satellite artificial chromosomes evidence Applicant's possession of the claimed subject matter.

The descriptions of identifying characteristics of satellite artificial chromosomes distinguish these chromosomes from wild-type endogenous chromosomes and other species of artificial chromosomes. For example, the specification describes that satellite artificial chromosomes contain more heterochromatin than euchromatin. An additional feature of satellite artificial chromosomes is their ability to carry heterologous DNA and an absence of other genetic information. As explained above, although satellite artificial chromosomes contain centromeres, telomeres and at least one origin of replication, they do not contain genes that might be potentially harmful or interfere when the chromosome is used as a vector. Hence, each of these features is an identifying characteristic that distinguishes satellite artificial chromosomes from other chromosomes known in the art and evidences Applicant's possession of the claimed subject matter.

Additionally, Applicant's possession of the claimed subject matter is demonstrated by the detailed figures depicting the structures of satellite artificial chromosomes. For example, Figures 2 and 3 depict the formation of satellite artificial chromosomes, including a megachromosome and intermediates. As noted above, these drawings were based on detailed studies of satellite artificial chromosomes, including *in situ* hybridization with satellite DNA (as a probe) for heterochromatic DNA, euchromatic probes and Hoechst staining and FPG staining to observe chromosome architecture within a satellite artificial chromosome. In addition, pulse field gel electrophoresis and southern hybridization were used to map satellite artificial chromosomes. Cloning and sequencing of regions of satellite artificial chromosomes were employed to confirm the structure of the chromosome. Additionally, the satellite artificial chromosomes were observed directly by scanning electron microscopy

(see, for example, pages 93-103 describing a detailed analysis of a megachromosome, an example of a satellite artificial chromosome).

The Examiner alleges that Figures 2 and 3 do not describe a satellite artificial chromosome structure because "[f]igures 2 and 3 show schematic of complex macromolecular pathway starting with mouse chromosome #7 being transfected with foreign DNA, which DNA is described as specific λ DNA. The other components are macromolecular complexes, comprising for example heterochromatin and euchromatin." Applicant respectfully submits that Figures 2 and 3 include explicit depictions of satellite artificial chromosomes. For example, Figure 3 at the bottom of the page shows a stable megachromosome, an exemplary satellite artificial chromosome (see pages 33-36 under section "Preparation of SATACs", which states that Figures 2 and 3 illustrate the production of SATACs). Figure 3 depicts the megachromosome with a centromere, telomeres, satellite DNA segments and tandem arrays of repeated chromosome segments. Each of these components is illustrated and labeled in Figure 3. Figure 2 illustrates the formation of artificial chromosomes including satellite artificial chromosomes. In particular, Figure 2F, depicts an exemplary satellite artificial chromosome (a megachromosome) with centromeric DNA, euchromatin, heterochromatin and integrated heterologous DNA. Again, each of these components is illustrated and labeled. A satellite artificial chromosome is a large molecule; it is a chromosome. Applicants have fully described its structure. Applicant respectfully submits that a description of a macromolecular structure, which evidences Applicants possession of satellite artificial chromosomes and provides distinguishing characteristics to identify such chromosomes from other chromosomes known in the art is sufficient to satisfy the written description requirement.

Applicant's possession of the claimed subject matter also is demonstrated by the detailed description of several cell lines, including G3D5, H1D3 and mM2C1 cell lines. Each of these cell lines contains a satellite artificial chromosome. Further, the satellite artificial chromosomes contained in the cell lines are described and characterized in detail (see for example, pages 36-39 and Example 6). Moreover, while not necessary for purposes of 35 U.S.C §112, first paragraph, G3D5 and H1D3 cells lines, as noted in the specification, are deposited with the ECACC under accession nos. 96040928 and 96040929, respectively (page 74, line 22 to page 75, line 7).

Although the Examiner alleges that deposit of a biological material can not overcome a written description rejection, such statement is incomplete. "An application specification may show reduction to practice by describing testing of the claimed invention or, in the case of biological materials, by specifically describing a deposit made in accordance with 37 C.F.R. 1.801 *et. seq.*" MPEP § 2163. Consistent with this statement, Applicant has not only deposited the material but fully described such material. For example, Example 6 (starting at page 93) describes in detail the cell lines and the satellite artificial chromosomes contained therein for cell lines G3D5 and H1D3. Thus, the written description is provided by the specification, and the deposits further attest to *and* demonstrate actual reduction to practice and possession of the claimed subject matter by the Applicant.

In summary, the detailed descriptions, figures and cell lines all demonstrate Applicant's possession of satellite artificial chromosomes as of the filing of the application. Moreover, these figures and accompanying descriptions are disclosed in the parent applications of the instant application, U.S. application serial nos. 08/695,191, 08/682,080 and 08/629,822, which are incorporated in their entirety by reference into the instant application, evidencing Applicant's possession of satellite artificial chromosomes as of the earliest effective filing date of the application.

The instant application also demonstrates possession of plant satellite artificial chromosomes as of the earliest filing date of the application. As noted in the "Response," the specification describes plant satellite artificial chromosomes. The specification describes that the methods provided therein for making satellite artificial chromosomes are applicable to plants. For example at page 9, lines 9-18, the specification states:

> Thus, methods for producing MACs of both types (*i.e.*, SATACS and minichromosomes) are provided. These methods are applicable to the production of artificial chromosomes containing centromeres derived from any higher eukaryotic cell, including mammals, birds, fowl, fish, insects and plants.
>
> The resulting chromosomes can be purified by methods provided herein to provide vectors for introduction of heterologous DNA into selected cells for production of the gene product(s) encoded by the heterologous DNA, for production of transgenic (non-human) animals, birds, fowl, fish and plants or for gene therapy.

In addition, the specification describes that plant satellite artificial chromosomes have the same structural elements as described for animal artificial chromosomes (see for example, at page 16, lines 22-29). The specification describes exemplary structural elements of

chromosomes, applicable to all species. These elements include centromeres, telomeres, an origin of replication and heterochromatin (see, for example, page 7, lines 15-20 and page 10, lines 12-19; see also, the section entitled "Identification and isolation of the components of artificial chromosomes" at pages 43-47 with subsections describing centromeres (page 44), telomeres (page 46), megareplicator (page 46,), filler heterochromatin (page 46) and selectable markers (page 47)).

For purposes of the 35 U.S.C. §112, first paragraph, written description requirement, t is not necessary that the disclosure describe the subject matter *in haec verba*. The written description requirement can be satisfied without express or explicit disclosure of the claimed subject matter. See *e.g.* In re Herschler, 591 F.2d 693, 700, 200 USPQ 711, 717 (CCPA 1979); Purdue Pharma L.P. v Faulding, Inc. 230 F.3d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000). ***The descriptions provided are descriptive of all satellite artificial chromosomes and demonstrate possession thereof.*** As noted above, the application indicates that characteristics of satellite artificial chromosomes described therein are applicable to all eukaryotic satellite artificial chromosomes. The specification explicitly states that plant satellite artificial chromosomes can be made by the methods therein. Thus, the detailed descriptions of structural and functional characteristics of satellite artificial chromosomes are directly applicable to plant satellite artificial chromosomes. Moreover, the satellite artificial chromosomes and cells containing satellite artificial chromosomes exemplified in the specification are representative of plant satellite artificial chromosomes, since, as described, the identifying characteristics are common to all satellite artificial chromosomes.

Applicant respectfully submits that the Examiner has not provided any basis to doubt that satellite artificial chromosomes provided in the application are representative of all satellite artificial chromosomes, including plant satellite artificial chromosomes. The application sets forth identifying characteristics that are not limited by species. The Examiner has provided no substantiated reasons why such identifying features are not common to all satellite artificial chromosomes, nor why such features are not sufficient to identify and show possession of plant satellite artificial chromosomes. The specification teaches that these characteristics are common to all species.

As noted above, an Applicant need not provide a representative of everything claimed, but may show possession by providing identifying features common to all members.

Applicant, by way of detailed descriptions of features, figures, working examples and exemplary satellite artificial chromosomes and cell lines, has done exactly that. Such disclosures evidence Applicants possession of plant satellite artificial chromosomes and cells containing such chromosomes. Therefore, it is respectfully submitted that Applicant had possession of the claimed subject matter at the time the instant application was filed as wells as at the time the parent applications were filed.

B. ***Satellite artificial chromosomes, their components and methods of producing them are sufficiently described in the instant application to "predictably" generate and identify plant or animal satellite artificial chromosomes as a starting material in methods such as the production of cells containing satellite artificial chromosomes.***

Although the issue of predictability is not relevant to a consideration of the adequacy of a disclosure in a specification, applicant notes that with respect to the allegation that satellite artificial chromosomes were not predictable as a starting material, the "Response" explains in great detail how animal and plant satellite artificial chromosomes are described in the instant application in a manner that predictably permits their production and introduction into a cell. For example, the "Response" notes how satellite artificial chromosomes are described in the instant application and exemplary satellite artificial chromosomes are provided, the elements of satellite artificial chromosomes are described and exemplary elements are provided, schematic representations of exemplary satellite artificial chromosomes are shown in the figures, and exemplary cell lines containing satellite artificial chromosomes are described and deposited with ECACC. These cells lines are described and their deposits disclosed in parent U.S. application serial no. 08/682,080. As noted above, during the prosecution of parent U.S. application serial no. 08/682,080, Applicant stated that all restrictions upon availability of the deposited material would be removed upon granting of a patent that included claims to the deposited material. Thus, upon issuance of U.S. Patent No. 6,077,697 with claims to such cell lines, the cell lines are available without restriction.

Further, with respect to predictability, the application provides methods for generating satellite artificial chromosomes that are amenable to any plant and animal species of satellite artificial chromosome and any plant and animal cell type. For example, the application discloses methods of introducing heterologous DNA with a selectable marker and/or sequences targeted to the pericentric region of a chromosome into a cell, growing cells under selective condition and selecting cells that have incorporated the DNA and contain a satellite artificial chromosome. Additionally, the application provides working examples of these

methods. Although the working examples are exemplified in animal cells, the application explains that methods of generating satellite artificial chromosomes are applicable to any plant and animal cell type and any plant and animal species of satellite artificial chromosomes (see for example, at page 9, lines 14-18, page 30, lines 9-12 and page 122, lines 25-26). The application provides descriptions of the elements of satellite artificial chromosomes and distinguishing features, such as a content of more heterochromatin than euchromatin. Further, the application provides methods for identifying satellite artificial chromosomes that are applicable to all cell types.

Therefore, notwithstanding the impropriety of applying "predictability" to an assessment of written description, the specification does in fact describe satellite artificial chromosomes and cells containing satellite artificial chromosomes in extensive detail so that satellite artificial chromosomes and cells containing satellite artificial chromosomes can be reproducibly and "predictably" identified.

Therefore, since the purpose of the written description requirement is to demonstrate that an Applicant was in possession of the invention at the time of the filing date sought. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991), the instant application provides sufficient description of relevant, identifying characteristics of satellite artificial chromosomes and plant satellite artificial chromosomes to evidence Applicant's possession of the claimed subject matter at the time of filing. As discussed above, satellite artificial chromosomes are described by their structural elements; they are chromosomes with the content of heterochromatin (greater than the euchromatin) and repetitive units of satellite DNA. Further, as discussed, the structural elements of satellite artificial chromosomes, are applicable to all eukaryotic satellite artificial chromosomes, including plant satellite artificial chromosomes.

The Office Action asserts that Applicant has not distinguished satellite artificial chromosomes from "wild-type" chromosomes. To the contrary, the specification explicitly points out the differences between the different types of chromosomes. For example, at page 17, lines 8-9, the specification states that endogenous chromosomes refer to genomic chromosomes as found in the cell before an artificial chromosome is generated. The specification then defines satellite artificial chromosomes (which are a type of artificial chromosome) as chromosomes that are contain more heterochromatin than euchromatin, composed of repeating units of short satellite DNA, and that contains more heterochromatin

than euchromatin (see, *e.g.*, page 7, lines 15-20, page 19, lines 4-6 and page 94, lines 3-21). The specification also describes that a satellite artificial chromosome can contain heterologous DNA in addition to heterochromatic nucleic acid (page 19, lines 4-6). Each of these is a feature that distinguishes it from an endogenous ("wild-type") chromosome. Endogenous chromosomes do not contain any heterologous DNA. Although endogenous chromosomes have regions of heterochromatin, for example at the centromere and telomeres, they are do not contain more heterochromatin than euchromatin (see for example Klug, W.S. and Cummings, M.R. (1983) Concepts of Genetics, pp. 201, provided herewith).

The Office Action further alleges that Applicant has neglected to describe an essential feature of satellite artificial chromosomes because Applicant has not described protein components of chromosomes. First it is noted, that the claims do not include such proteins as an essential element. Second, Applicant respectfully submits that Applicant is not required to describe what is already well-known in the art. Numerous publications in the art at the time of filing, such as those made of record in this application, describe protein components of chromosomes including histone and non-histone proteins. See, for example, Mole-Bajer *et al.* (1990) *Proc. Natl. Acad. Sci. USA 87*: 3599-3603; and Houben *et al.* (1995) *Chromosome Res. 3*: 27-31, already of record in this application. As discussed above, a satellite artificial chromosome is a chromosome with particular features. Thus, it shares characteristics with chromosomes, familiar to one of skill in the art. The application discusses protein components of chromosomes and how one of skill in the art can distinguish a satellite artificial chromosome from an endogenous chromosomes using such features. For example, at page 125, the specification describes the use of antibodies that bind to chromosomal proteins to separate satellite artificial chromosomes from endogenous chromosomes. Antibodies that bind condensed centromeric proteins or condensed and DNA-bound histone proteins are used to effect large scale isolation of chromosomes, prior to sorting of satellite artificial chromosomes by methods such as FACS. At page 126, the specification describes the use of antibodies that recognize heterochromatin proteins and their use in separating satellite artificial chromosomes. Hence, protein components are not lacking from applicants description of satellite artificial chromosomes.

Thus, it is respectfully submitted that Applicant has complied with the requirements of the written description requirement by describing the relevant, identifying structural features of satellite artificial chromosomes that are common to the genus.

C. ***Applicant had possession of the claimed subject matter as of the effective filing date as evidenced by the parent applications that have issued with claims to satellite artificial chromosomes and related compositions and methods.***

Applicant respectfully again points out that the instant application is a continuation-in-part of U.S. applications serial nos. 08/695,191, 08/682,080 and 08/629,822. Two U.S. patents have issued based on these applications: U.S. Patent Nos. 6,077,697 and 6,025,155. The Examiner alleges that the disclosure of satellite artificial chromosomes in the parent application that have issued as patents is not relevant because "each application is evaluated on its own merits;" this statement is however inapt with respect to the instant application. U.S. application Serial No. 08/695,191, 08/682,080 and 08/629,822 are the parent U.S. application for the issued U.S. Patent. Nos. 6,077,697 and 6,025,155. These applications are also the parent applications for the instant application. ***Thus, the issued patents and the instant application are part of the same patent chain and because the disclosure upon which the claims rely is the same are the same "case."***

The same disclosure provides basis for the claims in the instant case and in the patents that issued from the parent applications. The two issued patents, U.S. Patent Nos. 6,077,697 and 6,025,155 contains claims directed to methods of producing and isolating satellite artificial chromosomes, claims directed to isolated satellite artificial chromosomes, claims directed to cells containing satellite artificial chromosomes and claims drawn to cells containing satellite artificial chromosomes containing heterologous nucleic acid. As noted above, the claims of these two issued patents, U.S. Patent Nos. 6,077,697 and 6,025,155, find basis in U.S. application serial no. 08/629,822. ***The claims of the instant application also find basis in the same disclosure as the issued patents.*** The claims of the instant application find basis in U.S. applications serial no. 08/629,822, to which the instant application claims priority. Additionally, the disclosures of priority applications U.S. application serial nos. 08/695,191, 08/682,080 and 08/629,822 are incorporated by reference in their entirety into the instant application.

The claims of the issued patents include claims directed to satellite artificial chromosomes and cells containing satellite artificial chromosomes, not limited to any species. Claims in the issued patents are presumptively valid. 35 U.S.C. §282. As noted above, these claims find basis in U.S. applications serial no. 08/629,822. Hence, ***the Patent Office having issued claims*** in U.S. Patent Nos. 6,077,697 and 6,025,155, to satellite artificial chromosomes and cells containing satellite artificial chromosomes of any species based on

the disclosures of U.S. application serial no. 08/629,822, ***cannot now assert that the same disclosure is inadequate written description*** for claims to satellite artificial chromosomes and cells containing satellite artificial chromosomes. ***As set forth in 35 U.S.C. §282, an issued patent is presumptively valid and meets the requirements of written description. Furthermore, the MPEP states that the Examiner must give full faith and credit to determinations of the Patent Office and cannot denigrate the validity of an issued patent. MPEP §1701.*** Applicant therefore respectfully requests that the rejection be withdrawn.

III. The Rejection under 35 U.S.C. §112, first paragraph: Enablement

Claims 50-52 remain rejected under 35 U.S.C. §112, first paragraph, that, because the written description requirement allegedly is not met, one of skill in the art would not know how to make or use the claimed subject matter. Further, it is alleged that the specification, while being enabling for a mammalian satellite artificial chromosome in a mammalian cell, does not reasonably provide enablement for the introduction of a satellite artificial chromosomes from any source (*e.g.*, plants) that is operable in any cell type (*e.g.*, a plant cell). In particular, the Office Action alleges (1) that the differences between plants and animals, make it unpredictable that a plant would have centromeres that are structurally and biochemically the same as those of animals; and (2) that the alleged complexity of satellite artificial chromosomes, would lead one of skill in the art to expect that a satellite artificial chromosome constructed for mammalian cells would differ from a satellite artificial chromosome that is constructed for a plant cell.

In addition, regarding Declarations of Fabijanski, submitted July 16, 2003 (hereinafter Declaration 1), and April 22, 2004 (hereinafter Declaration 2), with the "Response", the Office Action alleges that the Declarations are unpersuasive because they allegedly do not show a SATAC in a transformed plant, the subject matter of the instant claims. Further, it is alleged that the Declarations do not use methods that are disclosed in the specification as filed. It also is alleged that the Declarations fail to provide guidance for growing a protoplast containing a SATAC into a transgenic plant. Therefore, the Office Action alleges that undue experimentation would be required to produce the claimed transgenic plants.

This rejection and all bases therefor are respectfully traversed.

Summary of Arguments

First, Applicant has rebutted the rejection for the alleged failure to provide an adequate written description. Therefore, since this rejection is premised on the written

description rejection, it is similarly obviated. Notwithstanding this fact, the following discussion as well as the Declarations of record and the Declaration provided herewith, demonstrates that the specification teaches how to make and use satellite artificial chromosomes, including plant satellite artificial chromosomes.

The *In re Wands* Analysis

In the Response, responsive to the previous Office Action, Applicant provided a complete and detailed analysis demonstrating that the instant application teaches one of skill in the art how to make and use satellite artificial chromosomes, including plant satellite artificial chromosomes without undue experimentation , and also how to make and use transgenic plants containing satellite artificial chromosomes without undue experimentation. Hence the teachings of the specification are commensurate in scope with the claims (the Examiner is referred, for example, to pages 21-44 of the "Response"). Employing an analysis of the issue based on the factors set forth in In re Wands, the "Response" directly addresses the issues raised by the Examiner, and demonstrates that: (1) the specification teaches one of skill in the art satellite artificial chromosomes can be transferred into and are operable in a broad variety of cell types; (2) the specification teaches one of skill in the art to prepare plant satellite artificial chromosomes and cells containing plant satellite artificial chromosomes; (3) employing methods taught in the specification and also well-known and established in the art, one of skill in the art could produce transgenic plants from cells containing SATACs. The analysis concludes that a consideration of the factors in In re Wands leads to the conclusion that undue experimentation would not be required to produce transgenic plants by introducing a satellite artificial chromosome (SATAC) into a plant protoplast and producing a transgenic plant as claimed.

Declarations

The conclusions of the analysis are further reinforced by Declarations 1 and 2 of Fabijanski, provided with the "Response." The Declarations demonstrate that by following the teachings of the application one of skill in the art can: (1) transfer satellite artificial chromosomes across species, including into plant cells; and (2) generate a plant satellite artificial chromosome and cells containing a plant satellite artificial chromosome.

The instant Final Office Action declares that the Applicant's remarks filed 22 April 2004 have been considered but are found unpersuasive. The instant Final Office Action, however, offers no explanation why the analysis provided by Applicant is unpersuasive.

Rather, the instant Final Office Action alleges that the two Declarations of Fabijanski submitted with the "Response" are not commensurate with the claims because they do not demonstrate production of a transgenic plant. The Final Office Action further alleges that no guidance is provided for growing a cell containing a satellite artificial chromosome into a plant.

Applicant respectfully submits that the In re Wands analysis in the "Response" addressed these issues. The "Response" demonstrated that sufficient knowledge and skill existed in the art at the time of filing such that one of skill in the art, provided with methods for making satellite artificial chromosomes and cells containing satellite artificial chromosomes set forth in the instant application and other disclosure in the application, could produce transgenic plants as claimed. The Declarations provided with the "Response" further evidenced that the teachings of the specification enable the introduction of a satellite artificial chromosome from any plant and animal source that in any plant and animal cell type. Applicant respectfully submits that by following the teachings of the application, one of skill in the art could make satellite artificial chromosomes and cells containing satellite artificial chromosomes that could then, by routine manipulations, be used to produce transgenic plants. Applicant has summarized these issues and addressed them below in more detail as follows:

1. *Generation of transgenic plants from plant cells was routine in the art at the time of filing*
2. *The specification teaches that satellite artificial chromosomes can be transferred into a broad variety of cell types*
3. *The specification teaches one of skill in the art to prepare plant satellite artificial chromosomes and cells containing plant satellite artificial chromosomes*
4. *The Declarations of Fabijanski further evidence that the teachings of the specification were enabling for the introduction of a satellite artificial chromosome from any plant and animal source in any plant and animal cell type*
5. *Policy Considerations: In light of Applicant's broad disclosure and pioneering discovery of satellite artificial chromosomes, it would be unduly limiting to restrict the scope of the instant claims.*

In addition, although not needed, Applicant submits here a Declaration of Dr. Fabijanski (herein after referred to as Declaration 3). Declaration 3 demonstrates that by following the teachings of the application and methods known in the art at the time of filing,

one of skill in the art can produce transgenic plants containing satellite artificial chromosomes.

DETAILED ANALYSIS

1. Generation of transgenic plants from plant cells was routine in the art at the time of filing

As noted above, the In re Wands analysis of the "Response" discussed the wide range of knowledge for making transgenic plants and the high level of skill of those practicing in the fields of molecular biology and transgenic plants. Generation of transgenic plants was routine in the art as of the earliest priority date of the instant application. Techniques for introducing DNA into a wide variety of plant cell types and plant species were known (and as noted in the previous response) detailed in the application . Methods for regenerating whole plants from plant cells and plant tissues were well known and routine in the art as well. One of skill in the art could use such information to produce transgenic plants as claimed.

Numerous techniques for introducing DNA into plant cells were known as of the earliest priority date of the instant application. The application lists (and incorporates by reference) numerous references including Uchimiya *et al.* (1989) *J. of Biotech. 12*: 1-20, Weissbach *et al.* (1988) *Methods for Plant Molecular Biology*, Academic Press, N.Y., Section VIII, pp. 421 463; Grierson *et al.* (1988) *Plant Molecular Biology*, 2d Ed., Blackie, London, Ch. 7 9, Miranda *et al.* (1992) *J. Bacteriol. 174*:2288-97 and for example, U.S. Patent Nos. 5,436,392; 5,489,520; 5,470,7085; 491,075; 5,482,928; and 5,424,409 and lists numerous methods (microprojectile bombardment, introduction into protoplasts, electroporation, among others).

Further each of the above-noted references, which are incorporated by reference into the specification, evidences the high level of knowledge and skill in the art for making transgenic plants. For example, Uchimiya *et al.* (1989) *J. of Biotech. 12*: 1-20, which is incorporated by reference into the application, reviews many of the available methods for introducing DNA including agrobacterium mediated transformation, and direct DNA transfer methods including PEG, electroporation, microinjection, and microprojectile bombardment. Uchimiya *et al.* also details the wide range of species that had been transformed as of the 1989 publication date of this reference, including rice, maize (corn), soybean, *Brassica napus*, lettuce, tomato, cotton, potato, poplar, tobacco, petunia and Arabidopsis. Uchimiya *et al.* not only sets forth plant species that have been transformed by foreign DNA, but also lists

the wide variety of species in which transgenic plants have been regenerated. Table 1 (pages 3- 5) lists approximately 20 exemplary species that were transformed using agrobacterium methods, types of genes used in the transformation and the dates of the publications which present the regenerated transgenic plants. Table 2 (page 6) lists about 10 exemplary species transformed by direct DNA transfer methods including electroporation, particle acceleration, liposome and PEG transfer. These species include rice, rye, carrot, petunia, tobacco and maize (corn). Uchimiya *et al.* notes that genetic transformation and gene transfer to plants is considered routine in the art (see, for example, page 14, final paragraph).

The routine nature of transformation and regeneration of plants also is evidenced by the commercial testing and sale of a plethora of transgenic plants as of the earliest priority date of the instant application. For example, Neumann (2000) *AgbioNet Proceedings 002*: Paper 12 (provided herein) reviews field trials and commercial sales of transgenic plants between 1990 and 1998. As shown in Figure 1, by 1995 (prior to the filing date of the instant application), there were 499 trials in Canada and 1926 trials in the United States involving transgenic plants. Each of these trials involved plants produced by transforming plant cells with DNA and regenerating transgenic plants from such cells. Figure 2 indicates that by 1996, greater than a million hectares had been planted worldwide with transgenic plants. ***Hence, transgenic plant production was routine as of the instant application's earliest filing date.***

The Examiner alleges that the instant application does not provide guidance for growing plants cells containing satellite artificial chromosomes into transgenic plants. It is further alleged that no information or guidance is given for the conditions, specific cells and types of explants needed to produce transgenic plants containing satellite artificial chromosomes. The Examiner provides no reasons why the conditions or cells types would be any different for cells containing satellite artificial chromosomes than for other cells that had been routinely employed to produce transgenic plants. Further, ***it is not necessary, however, for applicant to teach what is already known in the art.*** In re Buchner, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). ***It is sufficient if one of skill in the art can make and use the claimed subject matter using the teachings of the specification coupled with information known in the art.*** MPEP § 2164.01. Notwithstanding this, the

specification provides detailed guidance for preparing transgenic plants. For example, the specification at pages 54-55 states:

> transfer of DNA by processes, such as PEG-induced DNA uptake, protoplast fusion, microinjection, electroporation, and microprojectile bombardment [see, e.g., Uchimiya et al. (1989) J. of Biotech. 12: 1-20 for a review of such procedures, see, also, e.g., U.S. Patent Nos. 5,436,392 and 5,489,520 and many others] . . . .
>
> DNA uptake can be accomplished by DNA alone or in the presence of PEG, which is a fusion agent, with plant protoplasts or by any variations of such methods known to those of skill in the art [see, e.g., U.S. Patent No. 4,684,611 to Schilperoot et al.]. Electroporation, which involves high-voltage electrical pulses to a solution containing a mixture of protoplasts and foreign DNA to create reversible pores, has been used, for example, to successfully introduce foreign genes into rice and Brassica napus. Microinjection of DNA into plant cells, including cultured cells and cells in intact plant organs and embryoids in tissue culture and microprojectile bombardment [acceleration of small high density particles, which contain the DNA, to high velocity with a particle gun apparatus, which forces the particles to penetrate plant cell walls and membranes] have also been used. All plant cells into which DNA can be introduced and that can be regenerated from the transformed cells can be used to produce transformed whole plants which contain the transferred artificial chromosome. The particular protocol and means for introduction of the DNA into the plant host may need to be adapted or refined to suit the particular plant species or cultivar.

Hence the specification clearly describes production of transgenic plants. Also, as discussed above and also below, the specification details methods for making satellite artificial chromosomes and cells containing satellite artificial chromosomes. These teachings regarding plant satellite artificial chromosomes and plant cells containing satellite artificial chromosomes. Thus, as demonstrated by the wealth of literature and patent applications providing methods for making transgenic plants from plant cells, and the evidence of commercial trials and commercial sales of transgenic plants, as of the earliest filing date of the instant application, generation of transgenic plants from plant cells transformed with DNA was routine in the art. This knowledge coupled with the disclosure in the application adequately teaches those of skill in the art to prepare transgenic plants containing SATACs.

The Examiner alleges that because wild-type plants do not normally have SATACs, Applicant has provided no guidance regarding conditions, such as cells, plants, explants and types of SATACs that would be needed to make a transgenic plant. No basis for this conclusion is provided.

Applicant respectfully disagrees. First, as discussed above, generation of transgenic plants from a wide variety of cells, plants and DNAs was known and routine in the art. Secondly, also as pointed out above, satellite artificial chromosomes are chromosomes with particular distinguishing features such as more heterochromatin than euchromatic but that contain the requisite elements to function as chromosomes. Hence, satellite artificial chromosomes share characteristics with chromosomes *per se*. Knowledge of chromosome structure and behavior in plants was well known in the art at the time of filing. For example, it was known in the art before the time of filing that whole plants could be regenerated from plant cells following chromosome transfer. For example, Evans *et al.* (1980) *Physiol. Plant 48*:225-30, describes the methods of cells fusion to transfer chromosomes from one tobacco species into cells of another tobacco species. Whole plants were regenerated from the hybrid cells. The regenerated plants contained chromosomes from both species. Hence, chromosomes can be introduced into a plant cell and whole plants successfully regenerated from plants cells with extra chromosomes in a routine manner. In another example, Matzke *et al.* (1994) *Mol. Gen. Genet 245*:471-85, demonstrated that plants could be regenerated and stably maintained from cells containing extra chromosomes carrying heterologous DNA, including a kanamycin selectable marker and a GUS visible marker gene. In particular, the references show that plants regenerated from cells that carried an extra chromosome as compared to the wild-type chromosome number were morphologically normal and produced progeny plants that carried the extra chromosome. Hence, it was known in the art that plant cells containing an extra chromosome could be regenerated into whole plants using standard and routine methods known in the art. The Declaration provided herewith and discussed below, demonstrates this. Therefore, regeneration of plants cells containing an extra chromosome was available and routine in the art so that one of skill in the art could, by following the teachings of the application combined with the knowledge in the art, regenerate plants from plant cells containing a satellite artificial chromosome.

2. The specification teaches that satellite artificial chromosomes can be transferred into a wide variety of cell types

In the previous Office Action (October 22, 2003), the Examiner alleged that while the specification provides methods for the preparation and transfer of an animal satellite artificial chromosome into a mammalian cell, there is allegedly no evidence that these methods

produce a satellite artificial chromosome from any source that is operable in any cell type and therefore the claims are not commensurate in scope with the teachings of the application.

The Examiner provides no basis for these conclusions. In fact, contrary to the Examiner's assertion, the instant application teaches that satellite artificial chromosomes can be prepared in one cell type and transferred into a broad variety of cell types and they can be prepared in plants and animal cells. The In re Wands analysis of the "Response" (pages 26-44), describes in detail teachings of the instant application that are directed to the transfer of satellite artificial chromosomes into cells and the identification of such satellite artificial chromosomes within cells. ***These methods are not limited by the species of satellite artificial chromosome or species of cells.*** The In re Wands analysis includes a detailed presentation of the teachings of the specification that demonstrate detailed characterization of satellite artificial chromosomes and features of satellite artificial chromosomes applicable to a broad variety of species. The analysis, presented in the "Response," discusses the teachings of the specification that show how to characterize and isolate satellite artificial chromosomes and how to transfer satellite artificial chromosomes to a broad variety of cells from different species. The analysis describes the working examples demonstrating the preparation, identification and characterization and transfer of satellite artificial chromosomes. The analysis evidences the high level of skill in the art and knowledge in the art with respect to chromosomes and chromosomes structures from a broad variety of species, including plants. The analysis further evidences the predictability of the art, demonstrating that using the methods for preparation and characterization of satellite artificial chromosomes as taught in the application and the high level of knowledge in the art, one of skill in the art can prepare and transfer satellite artificial chromosomes into a broad variety of cell types.

The specification provides detailed information for the transfer of the artificial chromosomes into a broad variety of cells, including animal cells and plant cells (see, *e.g.*, page 9, lines 14-23; page 48, line 11, through page 51, line 26; page 54, line 1, through page 55, line 3; page 70, line 14, through page 72, line 27; and Examples 13 and 14 beginning on page 165). Such teachings are not limited to animal satellite artificial chromosomes. As discussed in detail in the "Response," the structure of chromosomes and elements of chromosomes, such as those found in satellite artificial chromosomes, are conserved among species and across kingdoms; centromeres, telomeres, origins of replication and

heterochromatin are recognizable structures across species. Further, as discussed more in detail below, such structural features of chromosomes from many species, including plants, were known in the art. Hence, as explained in the specification, the teachings of the specification are applicable to a broad variety of cell types and chromosomes. Furthermore, the Declarations of record and the Declaration provided herewith demonstrate this.

Applicant is not required to provide a specific example of everything within the scope of a broad claim. In re Anderson, 176 USPQ 331, at 333 (CCPA 1973). The requirements of §112, first paragraph can be fulfilled by the use of illustrative examples. In re Marzocchi et al., 469 USPQ 367 (CCPA 1971). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935). The instant application teaches the transfer of satellite artificial chromosomes from a wide variety of sources and into a wide variety of cell types and the preparation of satellite artificial chromosomes in a broad variety of cell types. The application provides illustrative examples of such teachings. Therefore, as discussed in the "Response," there was sufficient knowledge in the art regarding chromosomes and elements of chromosomes from a wide variety of species as of the application's effective filing date so that the teachings of the specification, when combined with such knowledge of those of skill in the art, are sufficient for the transfer of satellite artificial chromosomes to a wide variety of cell types, including plant cells.

In summary, the analysis shows that a consideration of the above factors and the other factors enunciated in In re.Wands leads inexorably to the conclusion that undue experimentation would not be required to introduce satellite artificial chromosomes into different cell types, including plant cells as claimed. In fact the Declarations of Fabijanski, discussed below, evidence this contention since they show the preparation of plant satellite artificial chromosomes, their introduction into plant cells and generation of transgenic plants.

3. The specification teaches one of skill in the art to prepare plant satellite artificial chromosomes and cells containing plant satellite artificial chromosomes

In the previous Office Action (October 22, 2003), the Examiner alleges that while the specification provides methods for the preparation of an animal satellite artificial

chromosome, it would be "unpredictable" to prepare plant satellite artificial chromosomes and cells containing plant satellite artificial chromosomes. Applicant respectfully disagrees.

Contrary to the Examiner's assertion, the instant application teaches the preparation of satellite artificial chromosomes for plants and animals. As discussed in detail in the In re Wands analysis of the "Response," the instant application provides a method for preparation of satellite artificial chromosomes per se. The method is applicable to any plant and animal species; it is not limited to the production of satellite artificial chromosomes from particular cells.

As shown in the In re Wands analysis, the teachings of the application are applicable to the preparation of satellite artificial chromosomes and cells containing satellite artificial chromosomes in a virtually any species of plant and animal (see for example, the application at page 9, lines 9-13). The specification teaches *de novo* satellite artificial chromosome formation and teaches identification and characterization of the satellite artificial chromosomes and intermediates that may occur in the process of forming satellite artificial chromosomes (see for example, at page 29, line 4 to page 30, line 12, page 39, line 25, through page 41, line 3; page 61, line 28, through page 62, line 7; page 150, line 1, through page 165, line 12 and Example 12 beginning on page 140). Further, as demonstrated in the In re Wands analysis, the knowledge in the art with respect to plant chromosomes and chromosome components was high. Thus, one of skill in the art could use such knowledge in combination with the detailed methods and identifying characteristics taught by the application to prepare plant satellite artificial chromosomes.

With respect to predictability, the In re Wands analysis demonstrates that one of skill in the art can prepare a plant satellite artificial chromosome using the teachings of the application in view of the knowledge in the art. For example, the specification teaches that by using heterologous DNA, one can produce dicentric chromosomes that are intermediates in the generation of any satellite artificial chromosomes, including plant satellite artificial chromosomes (see, for example, page 29, lines 11-17, and also Example 2). The ability of plants to form dicentric chromosomes was known in the art (see, for example, McClintock (1942) *P.N.A.S. 28*:458-63). Additionally, pericentric DNA that may be used as a targeting sequence was known for a variety of plants (see for example, Genbank Accession no. X52320; submitted 25 April 1990; Schmidt *et al. Science* (1995) 270:480-83; Murata *et al.* (1994) *Jpn. J. Genet. 69*:361-70). Selectable markers such as phosphinothricin acetyl

transferase and hygromycin for use in plants also were known (see, for example, White *et al.* (1990) *Nuc. Acids Res. 18*:1062; Spencer *et al.* (1990) *Theor. Appl. Genet. 79*:625-631; Vickers *et al.* (1996) *Plant Mol. Biol. Reporter 14*:363-368; Thompson *et al.* (1987) *EMBO J. 6*:2519-2523; and Blochinger and Diggelmann, *Mol. Cell. Biol. 4*:2929-2931).

In summary, as provided in the previous "Response," a consideration of the factors in In re Wands, including the teachings of the application, the scope of the claims, the level of skill in the art, the knowledge of one of skill in the art and the predictability of the subject matter, leads to the conclusion that undue experimentation would not be required to prepare satellite artificial chromosomes and cells with satellite artificial chromosomes, including plant satellite artificial chromosomes and plant cells with satellite artificial chromosomes as claimed.

4. The Declarations of Fabijanski further evidence that as filed the specification teaches one of skill in the art how to introduce a satellite artificial chromosome from any plant and animal source into any plant and animal cell type

In addition to the analysis of the factors In re Wands as discussed above that demonstrates the application teaches the transfer and identification of satellite artificial chromosomes from a wide variety of sources and into a wide variety of cell types, Applicant provided the Declarations 1 and 2 of Fabijanski (provided with the responses of July 16, 2003 and April 22, 2004, respectively) as further evidence that the teachings of the specification were enabling for the scope of the claims.

a. Declaration 1 evidences that satellite artificial chromosomes can be transferred into and are operable in a variety of cell types

Declaration 1 demonstrates that by following the teachings of the specification, cross-species transfer of satellite artificial chromosomes into cells can be effected between and among diverse species. Declaration 1 demonstrates that a SATAC prepared in one cell type (mouse) can be transferred, identified and stably maintained in various plant cells (tobacco, rice and *Arabidopsis* plant cells). Declaration 1 demonstrates that by following the teachings of the specification a mouse satellite artificial chromosome can be transferred from a mouse cell to a plant protoplast using microcell fusion. Declaration 1 demonstrates that satellite artificial chromosomes can be isolated from mouse cells and transferred to plant cells by lipid-mediated transfer.

The Examiner states that Declaration 1 is not persuasive because it does not show generation of a transformed plant. Applicant respectfully disagrees with such assertion. First, Applicant provided Declaration 1 to demonstrate that satellite artificial chromosomes from one cell type can be introduced and maintained in a other cell types. This issue had been raised by the Examiner in the previous Office Action mailed January 17, 2003. Declaration 1 demonstrates that the application teaches preparing transferring satellite artificial chromosomes across species and into plant cells. Thus, Declaration 1 addresses the issue raised by the Examiner in the previous Office Action. Second, as explained in detail above, generation of transgenic plants from plant cells is acknowledged by those of skill in the art to be routine (see Uchimiya *et al.* (1989) *J. of Biotech. 12*: 1-20). Hence, it is not necessary for Applicant to teach or demonstrate what is already well-known in the art.

The Examiner has provided no reason why one of skill in the art could not follow the teachings of the application to produce plants cells containing satellite artificial chromosomes, and then in view of the plethora of methods in the art for making transgenic plants to produce transgenic plants containing satellite artificial chromosomes. The Examiner appears to imply on page 10 of the instant Final Office Action, that specific conditions, cell types and tissue explants are necessary for generating such transgenic plants. The Examiner provides no reason why special conditions would be required. Applicant respectfully submits that no such requirements are set forth in the instant application, nor does the knowledge in the art at the time of filing suggest anything to the contrary. The Examiner has provided no supporting evidence to suggest that such requirements are evidenced by the art or the teachings of the application. Applicant has demonstrated, for example, in Declaration 1 that satellite artificial chromosomes can be transferred into tobacco, *Arabidopsis* and rice cells by following the teachings of the application and methods standard in the art. Plant regeneration from transformed plant cells was routine in the art as demonstrated by the wide variety of plant species transformed and the wide variety of methods and genes used in such transformations (see *e.g.*, Uchimiya *et al.* (1989) *J. of Biotech. 12*: 1-20). Thus, standard methods in the art could be used in the methods to make transgenic plants containing satellite artificial chromosomes as claimed.

b. Declaration 2 evidences that one of skill in the art by following the teachings of the specification can prepare plant satellite artificial chromosomes and cells containing plant satellite artificial chromosomes

Declaration 2 (submitted with the "Response" filed April 22, 2004) demonstrates that by following the teachings of the specification, one of skill in the art can produce a plant satellite artificial chromosome and identify and maintain a plant satellite artificial chromosome in a plant cell. Declaration 2 demonstrates element-for-element and step-for-step that, by following the teaching in the application, one can: (i) introduce heterologous DNA containing a selectable marker into a plant cell; (ii) grow the cell under selective conditions to produce plant cells that have incorporated the DNA into their genomic DNA such that a plant satellite artificial chromosome is produced; and (iii) select a cell that contains a plant satellite artificial chromosome.

In particular, Declaration 2 describes each of these points in detail. Declaration 2 demonstrates (i) introduction of heterologous DNA containing a selectable marker into a plant cell. Declaration 2 describes the introduction of heterologous DNA containing DNA with homology to the pericentric region of plant chromosomes, a selectable marker and a β-glucuronidase (GUS) reporter gene into tobacco cells. Declaration 2 demonstrates (ii) growing cells under selective conditions to produce plant cells that have incorporated the DNA into their genomic DNA such that a plant satellite artificial chromosome is produced. Declaration 2 describes the selection of cells on hygromycin and the selection of cells. The cells, as demonstrated by FISH analysis, incorporate heterologous DNA into plant chromosomes. The cells also have amplified vector DNA (heterologous DNA) and amplified pericentric regions (heterochromatic DNA). Declaration 2 also demonstrates (iii) selecting a cell that contains a plant satellite artificial chromosome. Declaration 2 describes selecting and identifying plant cells lines containing plant satellite artificial chromosomes, including a plant cell line with a plant satellite artificial chromosome that was maintained for over 6 months in culture. Thus, the Declaration 2 demonstrates the generation of plant cells containing plant satellite artificial chromosomes.

The Examiner, however, alleges that Declaration 2 is not commensurate in scope with the scope of the claims, because "none of the claims is drawn to a SATAC comprising plant amplified vector DNA as well as heterochromatic DNA." Contrary to this statement, the claims are drawn to methods for producing transgenic plants containing satellite artificial chromosomes. A satellite artificial chromosome, as described in the instant application, is a

chromosome that contains more heterochromatin than euchromatin and that can contain heterologous DNA. The Declaration describes plant cells containing a satellite artificial chromosome that includes amplified vector DNA and large scale amplification of pericentric regions. Plant vector DNA is an example of heterologous DNA. Amplified pericentric regions are an example of heterochromatic DNA (see for example, page 33, line 19 to page 34, line 7 of the application describing an example of generating a satellite artificial chromosome by targeting heterochromatic DNA, such as pericentric regions of a chromosome). Thus Declaration 2, in describing the generation of satellite artificial chromosome that includes heterologous DNA and large scale amplification of pericentric regions in plant cells, demonstrates the generation of plant satellite artificial chromosomes per se. Furthermore, as discussed in detail above, production of transgenic plants from plant cells was routine in the art. Hence, given the teachings of the specification for preparation of plant satellite artificial chromosomes and cells containing plant satellite artificial chromosomes, one of skill in the art could make transgenic plants containing plant satellite artificial chromosomes.

The Examiner also alleges that the methods used in Declaration 2 are not the methods described in the instant application. Applicant respectfully submits that the Examiner has provided no substantiation or reasoning to doubt the veracity of Declaration 2. The Declaration states that it follows the teachings of the application. The Declaration follows the methods described in the instant application, which include introducing DNA into a cell containing a selectable marker, pericentric DNA and a reporter gene (see, for example, page 6, lines 15-31, page 29, lines 11-12, page 33 line 20 to page 36, line 15). The Declaration further describes introducing the DNA using PEG-mediated transformation of tobacco, also described in the instant application (see for example, page 50, lines 16-23). Additionally, Declaration 2 describes FISH and reporter gene assays for identifying the satellite artificial chromosomes, also described in the instant application (see, for example, page 73, lines 5-16 and page 6, lines 15-31, page 29, lines 11-12). Hence, Applicant respectfully submits that Declaration 2 demonstrates that by following the teachings of the application one can produce and stably maintain plant satellite artificial chromosomes. Therefore, Applicant respectfully submits that Declarations 1 and 2 demonstrate that the teachings of the specification describe methods of producing transgenic plants with satellite artificial chromosomes, including plant satellite artificial chromosomes, as claimed.

As discussed above, regeneration of plants from transgenic plant cells was routine in the art. Applicant has demonstrated that the specification teaches one of skill in the art how to make satellite artificial chromosomes, including plant artificial chromosomes, and introduce artificial chromosomes into cells, including plant cells. Therefore, it would not require undue experimentation, given such teachings and the routine nature of regenerating plants from plant cells, to make transgenic plants containing a satellite artificial chromosome. In addition, although not needed, also provided herewith and discussed below, is a **Declaration of Fabijanski (Declaration 3)** demonstrating that by following the teachings of the application and using methods well-known and routine in the art for regenerating transgenic plants, one of skill in the art can make transgenic plants containing a satellite artificial chromosome.

(5) Policy Considerations: In light of Applicants broad and pioneering disclosure of satellite artificial chromosomes, it would be unduly limiting to restrict the scope of the instant claims.

The instant application provides broad teachings of a pioneering discovery, *i.e.* satellite artificial chromosomes, as of the application's earliest priority date. Prior to Applicant's disclosure, satellite artificial chromosomes were a heretofore unknown. Applicant is entitled to claims that are commensurate in scope not only with what Applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the Applicant has disclosed. The specification teaches the preparation and introduction of satellite artificial chromosomes that are not limited by species or cell type. Declarations 1 and 2 support the assertion of this breadth by demonstrating that by following the teachings of the specification one of skill in the art can: (1) transfer satellite artificial chromosomes across species, even among very diverse species, including transfer of a mouse satellite artificial chromosome to tobacco, *Arabidopsis* and rice; and (2) generate a plant satellite artificial chromosome and cells containing a plant satellite artificial chromosome. Moreover, the state of the art as of the earliest filing date clearly evidences the wealth of knowledge for making transgenic plants from a wide variety of plant cell types and plant species using a wide variety of available methods.

Therefore, it would be unfair, unduly limiting and contrary to the public policy upon which the patent laws are based to require Applicant to limit the instant claims to only the exemplified satellite artificial chromosomes or to specific cell types exemplified in view of the broad teachings of the application. To limit an Applicant to claims involving the specific

materials disclosed in the examples so that a competitor, seeking to avoid infringement can merely follow the disclosure and make routine substitutions "is contrary to the purpose for which the patent system exists - to promote progress in the useful arts"). See, *e.g.*, In re Goffe, 542 F.2d 801, 166 USPQ 85 (CCPA 1970).

> The public purpose on which the patent law rests requires the granting of claims commensurate in scope with the invention disclosed. This requires as much the granting of broad claims on broad inventions as it does the granting of more specific claims on more specific inventions" In re Sus and Schafer, 49 CCPA 1301, 306 F.2d 494, 134 USPQ 301, at 304.

To require Applicant to further limit the claims would permit those of skill in the art to practice what is disclosed in the specification but avoid infringing claims so-limited. To permit that is simply not fair. The instant application teaches the preparation of satellite artificial chromosomes from a broad range of species and the introduction of satellite artificial chromosomes into a broad range of cells. Having done so, it is now routine for others to prepare satellite artificial chromosomes from a broad variety species and to introduce them and identify them in cells of a broad variety of species. Those of skill in the art should not be permitted to make minor modifications and avoid infringing the instant claims.

Thus, given the broad teachings of the application, not limited by species of satellite artificial chromosomes or by species of cells containing satellite artificial chromosomes, Applicant is entitled to claims that reflect such broad teachings. For instance, by following the teachings of the application one of skill in the art could make satellite artificial chromosomes and plant cells containing satellite artificial chromosomes. Then, using techniques routine in the art, one of skill in the art could make transgenic plants containing satellite artificial chromosomes. To require Applicant to limit the claims so as not to cover transgenic plants, would permit one of skill in the art to follow the teachings of the application and with no more than routine manipulations, avoid infringing such claims when preparing a transgenic plant with a satellite artificial chromosomes. Such result would belie the public policy on which patent grants are based.

In summary, Applicant respectfully submits that the teachings of the specification for producing satellite artificial chromosomes and cells containing satellite artificial chromosomes, in view of the high level of the knowledge and skill in the art for producing

transgenic plants, do not require undue experimentation to achieve the methods of producing transgenic plants containing satellite artificial chromosomes as claimed.

DECLARATION OF FABIJANSKI

Notwithstanding the above arguments, to further evidence that the methods as claimed operate as claimed, attached is a Declaration (Declaration 3) under 37 C.F.R. 1.132 of Steven F. Fabijanski. Declaration 3 shows that by following the teachings of the application as of its earliest filing date, transgenic plants can be generating containing satellite artificial chromosomes.

It is noted that the level of skill in the biotechnical arts is recognized to be high (see, e.g., Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986). Further, methods for performing the various steps of the claimed methods, such as preparation of protoplasts, introduction of DNA into plant cells, techniques for the detection of specific DNA sequences (e.g., satellite artificial chromosomes) in recipient cells, and regeneration of transgenic plants from transgenic plant cells were known to the skilled artisan at the time of filing.

Dr. Fabijanski is not an inventor of this application. In performing or directing the experiments in Declaration 3, he followed the teachings in the application. Since those of skill in this art typically have advanced degrees, Dr. Fabijanski, who has an Ph.D. degree, is representative of a person of skill in this art with respect to performing experiments in accord with a disclosed protocol.

In particular, Declaration 3 demonstrates that by following the teachings in the application, transgenic plants containing satellite artificial chromosomes can be produced by i) introducing a DNA fragment with a selectable marker into a plant cell; ii) growing the cell under selective conditions to produce a plant cell that has incorporated the DNA into its genomic DNA, such that a plant cell containing a satellite artificial chromosome is produced; iii) growing the plant cell under conditions that regenerate a transgenic plant, such that a transgenic plant containing a satellite artificial chromosome is produced.

Heterologous DNA containing DNA with homology to the pericentric region of plant chromosomes, a selectable marker and a β-glucuronidase (GUS) reporter gene was introduced into tobacco cells. Cell lines (calli) that expressed the GUS reporter were selected. Fluorescence in situ hybridization (FISH) was used to demonstrate the formation of a sausage chromosome and a resulting plant SATAC. Callus from a cell line containing the SATAC was cultured on standard media to induce shoot and root formation. The shoots were

then transferred to soil and grown in the greenhouse. At least 20 transgenic plants were produced. These plants were morphologically normal, flowered and set seed. The results demonstrate that plant cells containing a satellite artificial chromosome can be regenerated into transgenic plants using standard methods, routine in the art. The regeneration methods followed methods published before the earliest filing date of the application, see *e.g.*, Robert *et al.* (1989) *Plant Molecular Biology 13*:399-309 (provided herein).

In addition, Declaration 3 demonstrates that by following the teachings in the application, transgenic plants can be produced that contain satellite artificial chromosomes by i) introducing a satellite artificial chromosome into a plant cell; ii) growing the protoplasts under conditions that regenerate a transgenic plant such that a transgenic plant containing a satellite artificial chromosome is produced. A satellite artificial chromosome was introduced into N. *glauca* cells by cell fusion with *N. tabacum* cells containing a satellite artificial chromosome. The transfer of the satellite artificial chromosome was confirmed by the expression of the β-glucuronidase marker and the hygromycin resistance marker present on the satellite artificial chromosome. The hybrid cells containing a satellite artificial chromosome were regenerated into transgenic plants using standard methods in the art. The hybrid cells were placed on media to induce shooting and rooting. The shoots were then transferred to soil and grown in the greenhouse. More than 50 regenerated plants were obtained. These experiments followed methods to regenerate plants from plant cells that are standard techniques, published before the earliest filing date of the instant application, including Evans *et al.* (1980) *Plant Physiology 48*: 25-230 and Robert *et al.* (1989) *Plant Molecular Biology 13*:399-309 (provided herein).

Therefore, Declaration 3 demonstrates that the specification, in light of the knowledge of the art at the time of filing, teaches one of skill in the art how to make transgenic plants containing a satellite artificial chromosome without undue experimentation. The Declaration demonstrates element-for-element and step-for-step that, by following the teaching in the application, transgenic plants can be generated that contain satellite artificial chromosomes by i) introducing a DNA fragment with a selectable marker into a plant cell; ii) growing the cell under selective conditions to produce plant cells that have incorporated the DNA into their genomic DNA and where the DNA undergoes amplification, such that a plant cell containing a satellite artificial chromosome is produced; iii) growing the plant cell under conditions that regenerate a transgenic plant such that a transgenic plant containing a satellite

artificial chromosome is produced. Declaration 3 further demonstrates that transgenic plants can be produced that contain satellite artificial chromosomes by i) introducing a satellite artificial chromosome into a plant cell; and ii) growing the protoplasts under conditions that regenerate a transgenic plant, such that a transgenic plant containing a satellite artificial chromosome is produced.

Applicant also notes that all of the experiments described in the Declarations (Declarations 1, 2 and 3) follow the teachings as disclosed in the instant application and in the parent applications, U.S. Patent Nos. 6,025,155 and 6,077,697, and U.S. application serial nos. 08/835,682 and 08/629,822. The Declarations demonstrate the use of DNA targeted to the pericentric region of the chromosome for the generation of a plant SATAC, the introduction of SATACs into plant cells and the production of transgenic plants, all of which are taught by the instant application and the parent applications. In light of these remarks, Applicant respectfully requests that the arguments presented in the previous "Response" as well as the Declarations of Fabijanski be reconsidered along with the remarks herein and the further Declaration and that the rejection be withdrawn.

* * *

In view of the above, examination of the application on the merits and allowance is respectfully requested.

Respectfully submitted,

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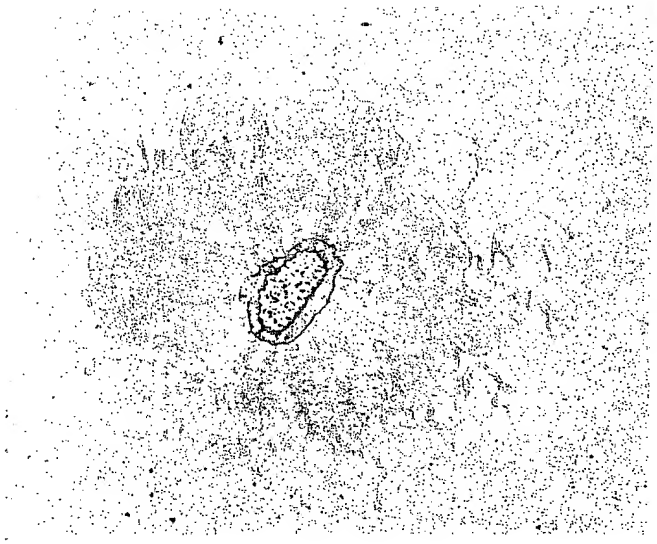


FIGURE 10.4
Electron micrograph of the bacterium *Hemophilus influenzae*, which has had its DNA released by osmotic shock. The chromosome is about 830 μm long. [With permission from MacHattie, 1965, p. 648. Copyright: Academic Press Inc. (London) Ltd.]

Escherichia coli, the most extensively studied bacterium, has a DNA molecule 1200 μm (1.2 mm) in length. This molecule exists in a circular form, similar to that of certain viruses. Table 10.1 compares the dimensions of bacterial DNA with those of the viral nucleic acids and shows that bacterial DNA is generally much longer. These cells, like viruses, also face a packing problem. In the case of bacteria, however, the genetic material is not functionally inert, even though it is somewhat compacted. Thus, in this condition, transcription of the genetic information may occur quite readily.

EUKARYOTIC CHROMOSOMES: GROSS STRUCTURE

The structure and organization of the genetic material in **eukaryotic cells** is much more intricate than in viruses or bacteria. This complexity is due to the total amount of DNA per chromosome and the presence of large numbers of proteins associated with DNA in eukaryotes. For example, while DNA in the *E. coli* chromosome is 1200 μm long, the DNA in human chromosomes ranges from 14,000 to 73,000 μm in length as a linear duplex. In a single human nucleus, all 46 chromosomes contain sufficient DNA to cover a length measuring almost 2 m. This genetic material, along with its associated proteins, is contained within a nucleus which usually measures about 5 μm in diameter!

For many reasons this intricacy is to be expected. It parallels the structural and biochemical diversity of the many types of eukaryotic cells making up a single organism. In a single multicellular organism, cells assume specific functions by "division of labor." It is assumed that any functional capability is based upon biochemical activity. Since this activity is under the direction of the genetic information, a highly ordered regulatory system governing the readout of this information must exist if dissimilar cells are to perform a variety of different functions. Such a system must in some way be imposed on or related to the molecular structure of the genetic material.

While bacteria can reproduce themselves, they never exhibit a detailed process similar to mitosis. As was pointed out in Chapter 2, eukaryotic cells exhibit a highly organized cell cycle. During interphase, the genetic material is finely dispersed throughout the nucleus in chromatin form. As mitosis begins, the chromatin condenses greatly, and during prophase it is compressed into recognizable chromosomes. This condensation represents a contraction in length of some 10,000 times for each chromatin fiber. This highly regular condensation-uncoiling cycle poses special organizational problems in eukaryotic genetic material.

MITOTIC CHROMOSOMES

Initially, biologists knew of eukaryotic chromosome structure only from observations made with the light microscope. Most information came from studies of mitotic chromosomes. By observing preparations of mitotic chromosomes from different species, geneticists and cytologists are able to determine the diploid number characteristic of any species and to note the varying sizes and gross morphology of chromosome pairs.

By the metaphase stage of mitosis it becomes apparent that each chromosome is really a double structure consisting of two **sister chromatids.** Sister chromatids are held together at a single point, the **centromere,** which is the area of attachment to the spindle fibers. Chromosomes for any given species are classified by the location of the centromere and the overall size of the chromosome. The **karyotype** consists of a micrograph of the chromosome pairs in metaphase arranged by size and centromere location. The number of chromosome pairs in the karyotype is equal to the haploid number. In species with a low haploid number, each pair of chromosomes may be distinct in gross morphology from all other pairs.

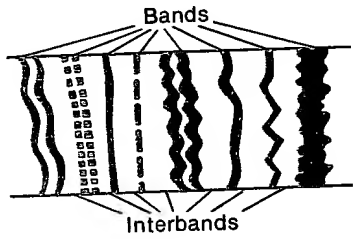
In humans, however, there is considerable morphological similarity between some pairs. In an initial classification scheme, human mitotic chromosome pairs were divided into seven categories (A–G), determined by size and by centromere position. The centromere position establishes the arm lengths on either side of the centromere (see Figure 2.3). This scheme is called the **Denver classification system** because it was established at a conference held in that city in 1960. Since then, revised procedures for staining mitotic chromosomes have been devised which result in **chromosome banding.** The various patterns observed allow all 23 human chromosomes to be distinguished from one another. We will discuss this topic later in this chapter.

The study of mitotic chromosomes and karyotypes has been important in several genetics disciplines. The way in which chromatin folds up into mitotic chromosomes is of interest to students of structure. **Chromosome mutations** or **aberrations** (abnormal numbers or arrangements of chromosomes) may be detected in karyotypes and linked to human disorders. The evolutionary relationships between different species may be gauged by similarities between chromosome number and morphology.

POLYTENE CHROMOSOMES

Studies of other unique chromosomes with the light croscope have yielded additional information about g chromosome morphology. Cells from a variety of ganisms contain large **polytene chromosomes.** They found in various dipteran larval cells (salivary, mid rectal, and malpighian excretory tubules) and in sev species of protozoans and plants. The large amour information obtained from studies of these chrc somes has provided a model system for more re investigations. Such structures were first observe Balbiani in 1881 and are illustrated in Figure 10.5.

The giant size and distinctiveness of such chr somes result from the many DNA strands that com them. Many replications occur without strand se tion or cytoplasmic division. As replication proceed homologues remain paired, ultimately creating mosomes having 1000 to 5000 DNA strands each i



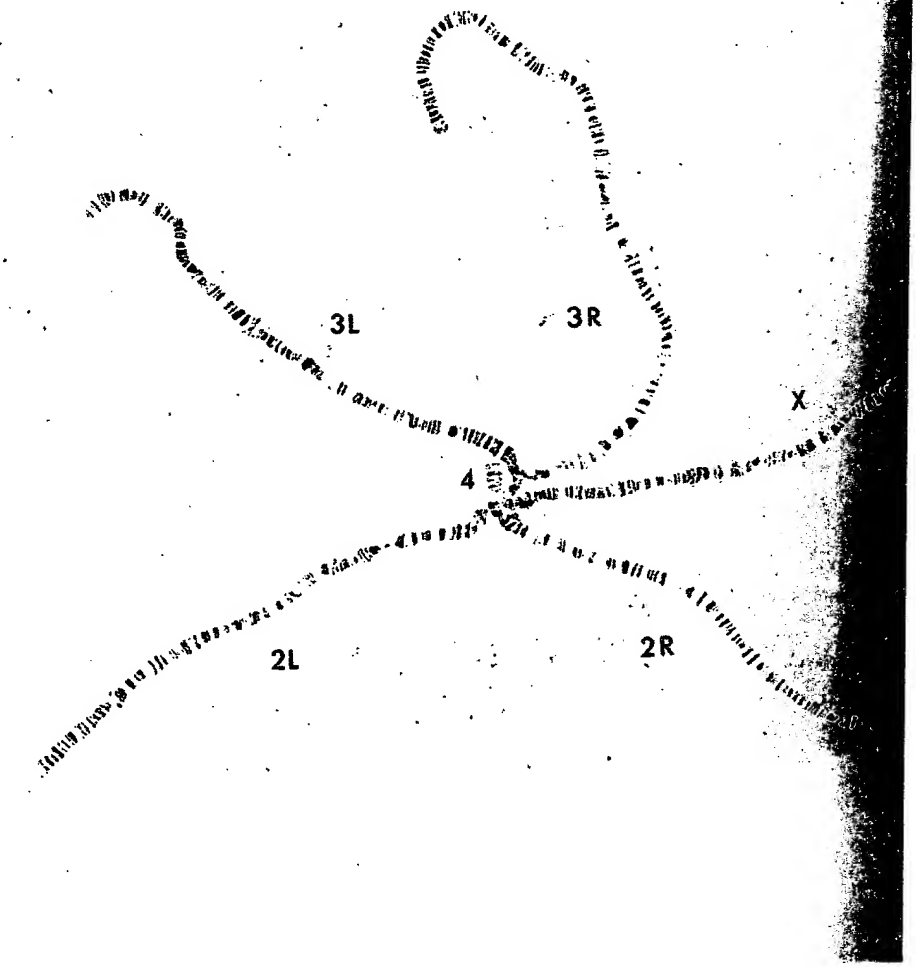


FIGURE 10.5
Giant polytene chromosomes derived from salivary gland cells of *Drosophila melanogaster*. The X chromosome, the right and left arms of chromosomes 2 and 3, and the small chromosome 4 are seen projecting from the chromocenter, where the centromeres of all chromosomes are connected. The insert depicts bands and interband regions along the axis of each chromosome. [With permission from Lefevre, 1976. Copyright: Academic Press Inc. (London) Ltd.]

allel register with one another. In conjunction with associated chromosomal proteins, giant chromosomes are created. Their parts may be more easily studied than those comprising a haploid interphase chromosome because of their visibility.

When polytene chromosomes are observed under the light microscope, each is seen to be composed of a linear series of **bands** and **interbands** (see insert in Figure 10.5). The banding pattern is distinctive for each homologous pair of chromosomes in any given species. Individual bands are sometimes called **chromomeres,** a more generalized term which describes lateral condensations of material along the axis of a chromosome. Each polytene chromosome is 200 to 600 μm long. Each of the 1000 or more fibers is a continuous DNA double helix with associated protein.

The genetic information, arranged as a linear series of bands, is contained in these fibers. In order for any particular gene, represented by all or part of a band, to become active in transcription, a localized uncoiling usually occurs. As the DNA unreels, the enzymes essential to transcription have access to it. A diagrammatic interpretation of this description is shown in Figure 10.6. Such localized sites of genetic activity are referred to as **puffs** because of their appearance.

Until recently it has been unclear what, if any, differences exist in the DNA of band and interband regions. However, following its discovery in 1979 (see Chapter 8), Z-DNA has been shown to be present preferentially in the interband regions of polytene chromosomes in *Drosophila melanogaster*. By using fluorescent antibodies made against Z-DNA, Alexander Rich and colleagues have shown that different staining intensities occur in these regions (Figure 10.7).

Rich's observation is important from several standpoints. First, it demonstrates that Z-DNA is a normal component of chromosomes, not just a conformation induced under laboratory conditions. Second, while the significance of this difference is not yet clear, the restriction of Z-DNA to interband regions prompts several interesting speculations. The presence of Z-DNA in these regions, traditionally thought to be devoid of genes, may be related to the control of chromosomal replication and/or gene activity.

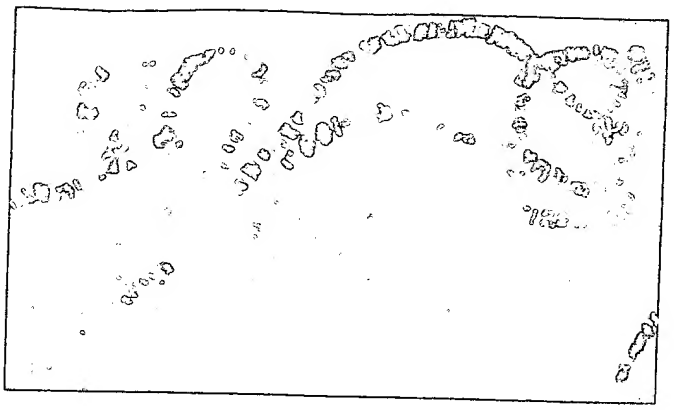
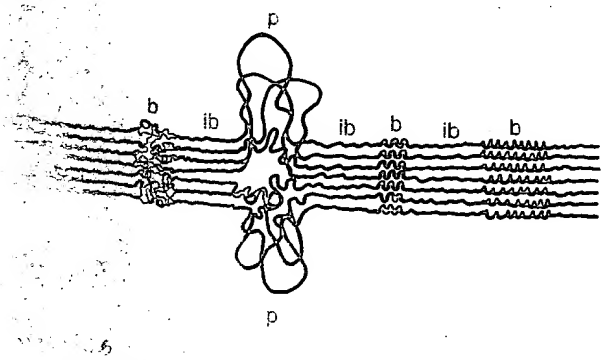


FIGURE 10.7
Fluorescent micrograph showing how antibodies prepared against Z-DNA bind to polytene chromosomes of *Drosophila melanogaster*. Specific binding has occurred only in the areas that appear white. These areas are exclusively interband regions. (From Nordheim et al., 1981. Reprinted by permission from *Nature*, Vol. 294, pp. 417–22. Copyright © 1981 Macmillan Journals Limited. Photo supplied by Alexander Rich.)



...presentation of the uncoiling of strands within ...ion to produce a puff (p) in polytene ... Interband regions are labeled ib.

CYTOLOGICAL MAPPING

By the early 1930s **genetic** or **linkage maps,** which gave the sequence and distances between many genes on the chromosomes of *Drosophila,* had been worked out (see Chapter 6). In that decade, these linkage maps were successfully correlated with the order of bands seen on the polytene chromosomes of *Drosophila*. This was accomplished by studying the altered banding patterns produced by flies with **chromosomal aberrations.** Aberrations (see Chaper 12) include duplications, deletions, and other rearrangements of the chromosomal material. In flies that demonstrated both a mutant phenotype and an aberration detectable in polytene chromosomes, the mutation was assigned to a region or band along the chromosome. In this way, a **cytological map** of many mutations on the *Drosophila* polytene chromosomes was compiled. A part of the *Drosophila* cytological map and its correlation with genetic loci identified by linkage studies are shown in Figure 10.8. Both the linkage and cytological maps are in agreement on

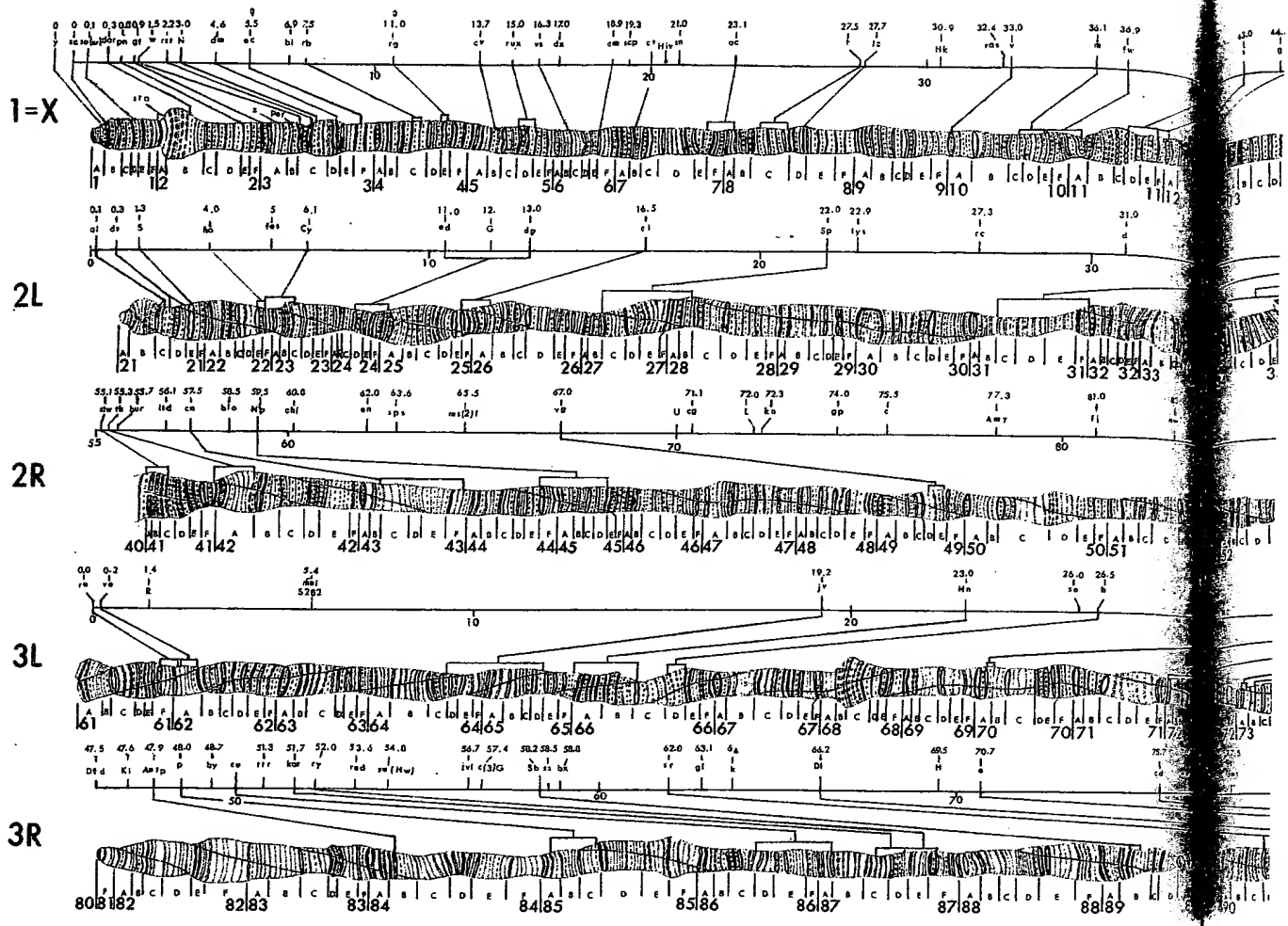
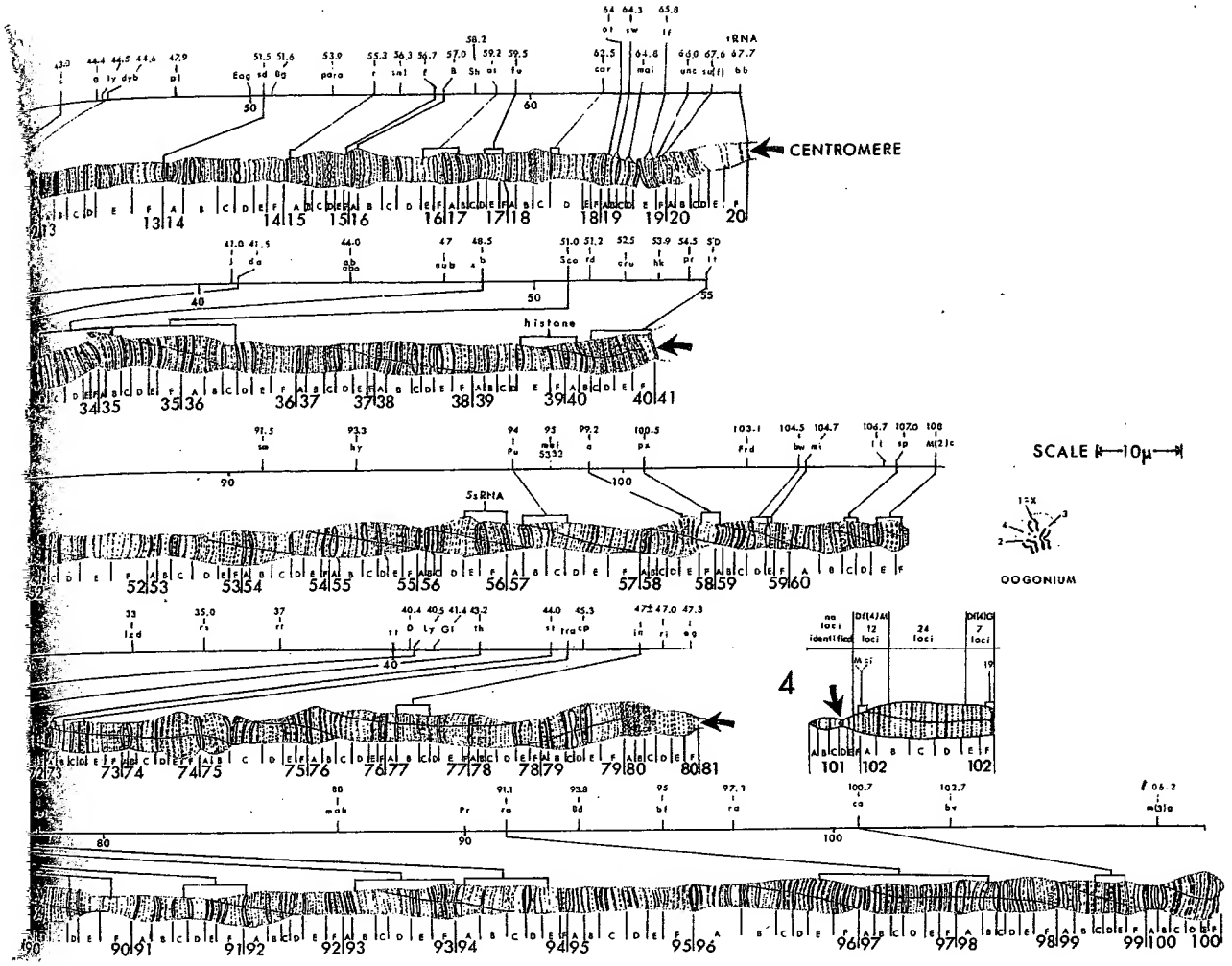


FIGURE 10.8
Comparison of the cytological map and the genetic map of the chromosomes of *Drosophila melanogaster*. The large designations at the left indicate the chromosome number and the right or left arm. The cytological map has been devised from the polytene chromosomes of larval salivary gland cells. It is divided into 102 major divisions, each of which is subdivided into six lettered subdivisions (A–F). The genetic map and many gene sites are shown above each cytological map. (From King, 1975, Fig. 1.)

the sequence of genes. However, the relative distance between any two loci as determined by linkage mapping and cytological techniques differs considerably in many instances.

For many years, it was postulated that each band on the *Drosophila* chromosomes represented one gene. Geneticists counted approximately 5000 bands on the four chromosomes of this species, and this number seemed to agree quite well with the estimated number of genes in *Drosophila*. More current information, however, has led geneticists to question whether there are not, in fact, more than 5000 genes. For example, there is sufficient DNA on the X chromosome alone to represent approximately 30,000 genes if each is composed of 1000 nucleotide pairs. Each of the 1000 chromomeres or bands on the X chromosome could represent as many as 30 genes.

Burke Judd and colleagues have extensively examined a small region of the *Drosophila* X chromosome consisting of about 15 bands. They induced and analyzed over 100 mutations which mapped in this general region. Careful analysis revealed that the mutations fell into **complementation groups,** the number of which was equivalent to the number of bands in the region studied. A complementation group (see Chapter 19) is the equivalent of one functional genetic unit, or gene. When



each complementation group was assigned to a particular band, it was concluded that a single band probably contains only a single gene. If this is true, and if each band contains sufficient DNA to code for 20 to 30 genes, what role is played by the remaining DNA?

Judd's work in the early 1970s suggested two possible roles for the remaining DNA which are not mutually exclusive. Perhaps the eukaryotic gene contains much more DNA than predicted by the size of an average protein. If so, what is the role of noncoding DNA contained within a gene, and what is its fate during transcription and translation? Perhaps the majority of eukaryotic DNA is noncoding and interspersed between classical genes. If so, what is its role in genetic processes? As we will see, both roles have been shown to be correct. In the former case, genes have been shown to contain nucleotide sequences which are transcribed but spliced out of mRNA before translation. (This topic is discussed in detail in Chapter 19.) In the second case, as we will see later in this chapter, noncoding repetitive DNA occupies a variable portion of eukaryotic genomes.

Studies of polytene chromosomes have provided valuable insights into other areas of genetics. For example, polytene chromosomes are used in studies of chromosomal aberrations (see Chapter 12), develop-

mental genetics (see Chapter 21), and evolution-population genetics (see Chapters 24 and 25).

LAMPBRUSH CHROMOSOMES

Another type of chromosome that has provided insights into chromosomal structure is the **lampbrush chromosome.** It was given this name because it is similar in appearance to the brushes used to clean lamp chimneys in centuries past. Lampbrush chromosomes were first discovered in 1892 in sharks and are now known to be characteristic of vertebrate oocytes as well as spermatocytes of some insects. Most experimental work has been done with material taken from amphibian oocytes.

These unique chromosomes are easily isolated from oocytes in the first prophase of meiosis, where they are active in directing the metabolic activities of the developing cell. The homologues are seen as synapsed pairs held together by chiasmata, but instead of condensing, as do most meiotic chromosomes, the lampbrush chromosomes often extend to combined lengths of 800 μm. Figure 10.9(a) shows the meiotic tetrad configuration of these chromosomes; Figure 10.9(b) shows that the linear axis contains large numbers of repeating condensations. As in polytene chromosomes, these segments are called chromomeres. Each chromomere may support a pair of **lateral loops,** which give the chromosome its distinctive appearance.

The structure and the functional activities of lampbrush chromosomes have been studied with enzymatic digestion and radioactive tracer techniques. Although considerable amounts of RNA and protein are associated with the chromosome, neither RNase nor protease enzymes disrupt the linear integrity of either the axis or the loops. It has been concluded, therefore, that DNA provides the skeletal structure of the chromosome which is associated with protein and RNA.

Each loop [Figure 10.9(c)] is thought to be composed of one double helix, and the central axis is made up of two DNA helices. This hypothesis is consistent with the belief that each chromosome is composed of a pair of sister chromatids. When oocytes are incubated in the presence of radioactive RNA precursors, the sites of transcription may be determined by autoradiographic analysis. Such studies reveal that the loops are active in the synthesis of RNA. The lampbrush loops, similar in a way to puffs in polytene chromosomes, represent DNA which has been reeled out from the central chromomere axis during transcription. As with polytene chromosomes, the study of lampbrush chromosomes has provided many insights into the arrangement and function of the genetic information.

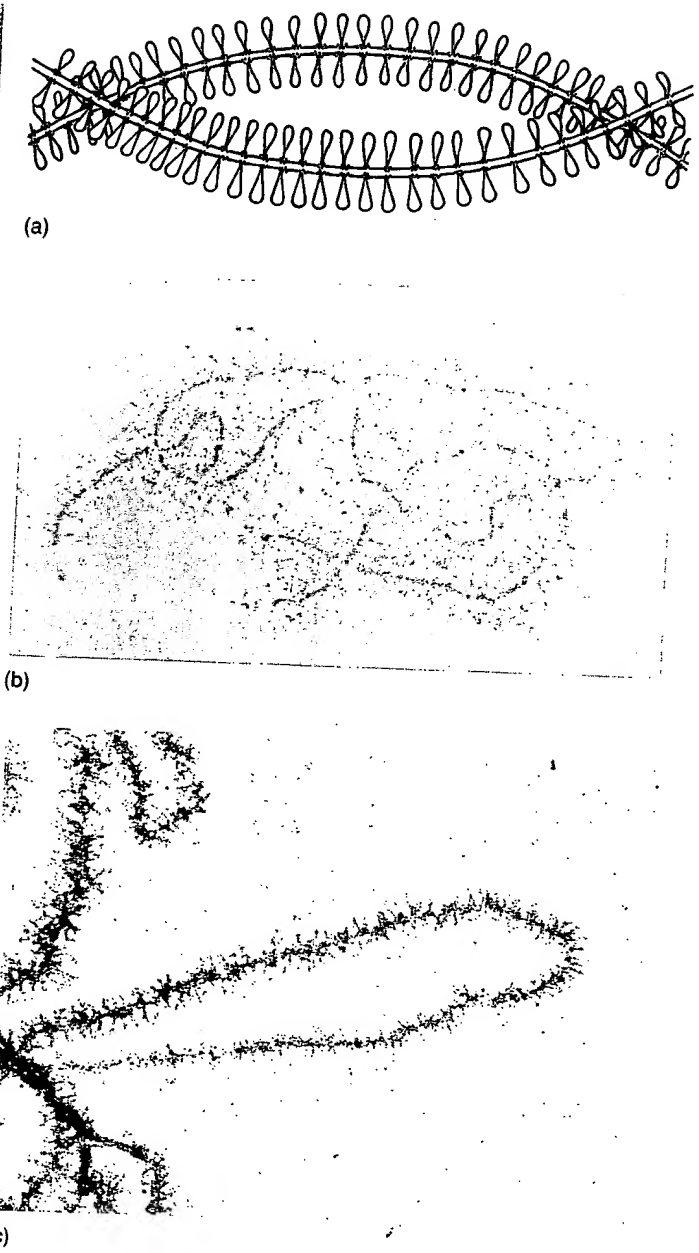


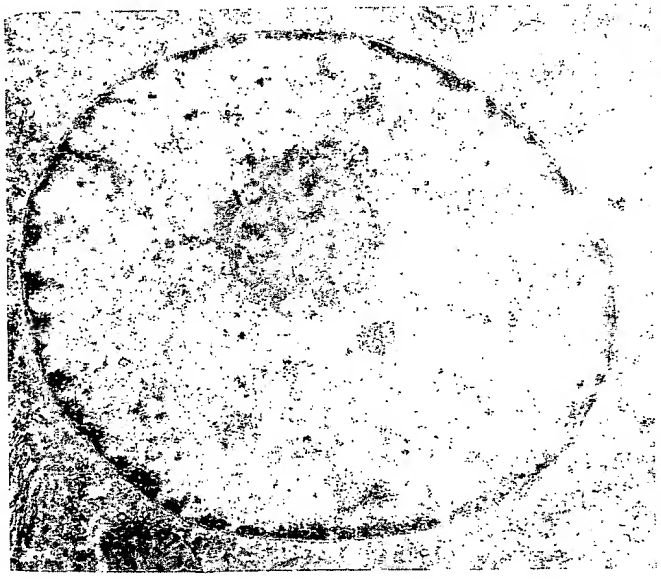
FIGURE 10.9
Lampbrush chromosomes. (a) Schematic drawing of the tetrad nature of a single lampbrush chromosome. (b) Photomicrograph from the oocyte nucleus of the newt *Notophthalmus viridescens* viewed under phase contrast. (Courtesy of Joe Gall.) (c) Electron micrograph of several loops extending from the axis of a lampbrush chromosome which shows the RNA and protein matrix covering each loop. (From Miller, 1965, p. 79.)

EUKARYOTIC CHROMOSOMES: MOLECULAR ORGANIZATION

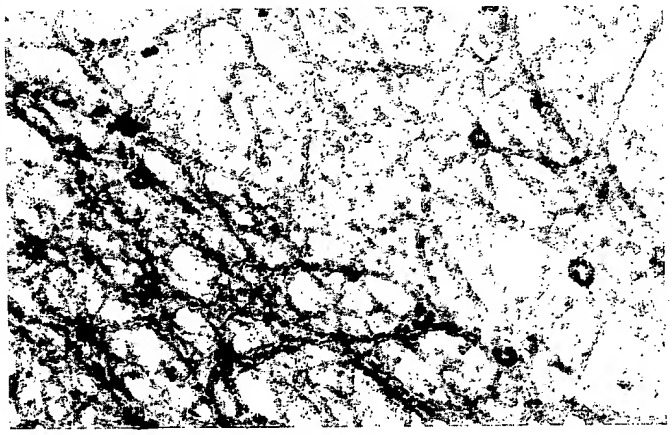
Early studies of the structure of eukaryotic genetic material concentrated on intact chromosomes, preferably large ones, because of the limitation of light microscopy. The development of techniques for biochemical analysis, as well as the examination of relatively intact eukaryotic chromatin and mitotic figures under the electron microscope, have greatly enhanced our understanding of chromosome structure. Recently, a combination of these techniques has been used to elucidate the organization of genetic material contained in the mitochondrion and the chloroplast.

CHROMATIN COMPOSITION AND STRUCTURE

As established earlier, the genetic material of viruses and prokaryotic cells takes the form of naked strands of DNA or RNA. This is not the case in eukaryotic chromosomes. Instead, a substantial amount of protein is associated with the chromosomal DNA in all phases of the eukaryotic cell cycle. Thus, the eukaryotic genetic material is composed of **nucleoprotein;** such material is generally referred to as **chromatin,** particularly during interphase, when it is uncoiled. The associated proteins are divided into basic, positively charged **histones** and less positively charged **nonhistones.** The histones seem to be intimately associated with chromatin structure, while the nonhistone proteins are thought to play other roles, including genetic regulation. Despite the presence of protein in the chromatin, the DNA component is universally believed to be the part that stores genetic information.



(a)



(b)

(c)

FIGURE 10.10
Eukaryotic chromatin seen in different ways. (a) Electron micrograph of a thin section cut through a mouse liver nucleus. Because the fibers have been sectioned, they appear as the lighter specks. The dense circular structure is the nucleolus. (b) Whole-mount electron micrograph of chromatin fibers derived from a mouse liver nucleus. The circular structures are nuclear pores. (c) Model depicting the supercoiling which is thought to occur and cause condensation of the chromatin fiber. (Micrographs courtesy of David E. Comings and Tadashi A. Okada.)

Research in the past several years has made it possible to develop a general model for chromatin structure (Figure 10.10). This model is based on the assumption that chromatin fibers, composed of DNA and protein, must undergo extensive coiling and folding in order to fit into the cell nucleus.

Of the proteins associated with DNA, the histones are now believed to be essential to the structural integrity of chromatin. Histones contain large amounts of the positively charged amino acids lysine and arginine. Thus, histones can bond electrostatically to the negatively charged phosphate groups of nucleotides. There are five main types of histones (Table 10.2); together in chromatin, they exist in a 1:1 mass ratio with DNA.

X-ray diffraction studies show that histones play an important role in chromatin structure. Under such investigation, chromatin produces regularly spaced diffraction rings, suggesting that repeating structural units occur along the chromatin axis. If the histone molecules are chemically removed from chromatin, the regularity of the diffraction rings disappears. In 1970, John Pardon and his colleagues proposed that the DNA-histone complex would conform to the observed X-ray diffraction pattern if it were twisted into a supercoiled helix, an idea suggested earlier by other workers. Such a supercoiled complex is illustrated in Figure 10.10(c). In such a model, the basic DNA-histone fiber is approximately 30 Å in diameter. Supercoiling can increase this measure to 100 to 200 Å.

This basic model for chromatin has been refined recently. Between 1973 and 1977, observations from a large number of workers in many laboratories served as the basis for the development of a general model for chromatin. Together these observations may well be regarded as a significant breakthrough in this discipline:

1. Digestion of chromatin by certain endonuclease enzymes, such as micrococcal nuclease, yields DNA fragments that are approximately 200 base pairs in length or multiples thereof. Such work was reported by D. Hewish and L. Burgoyne in 1973 and subsequently by others. These data suggest that enzymatic digestion is not random, for if it were, we would expect a wide range of fragment sizes. They also suggest that chromatin consists of repeating units, each of which is protected from enzymatic cleavage except where any two units join each other. Thus, the area between all units is attacked and cleaved by the nuclease. Such information agrees with the results of X-ray diffraction studies, which suggest regular spacing arrangements in chromatin.
2. In 1974, Ada and Donald Olins reported on electron microscopic observations of chromatin prepared by methods different from those of most earlier work.

TABLE 10.2
Categories and properties of histone proteins.

Histone Type	Lysine-Arginine Content	Molecular Weight (daltons)
H1	Lysine-rich	21,000
H2A	Slightly lysine-rich	14,500
H2B	Slightly lysine-rich	13,700
H3	Arginine-rich	15,300
H4	Arginine-rich	11,300

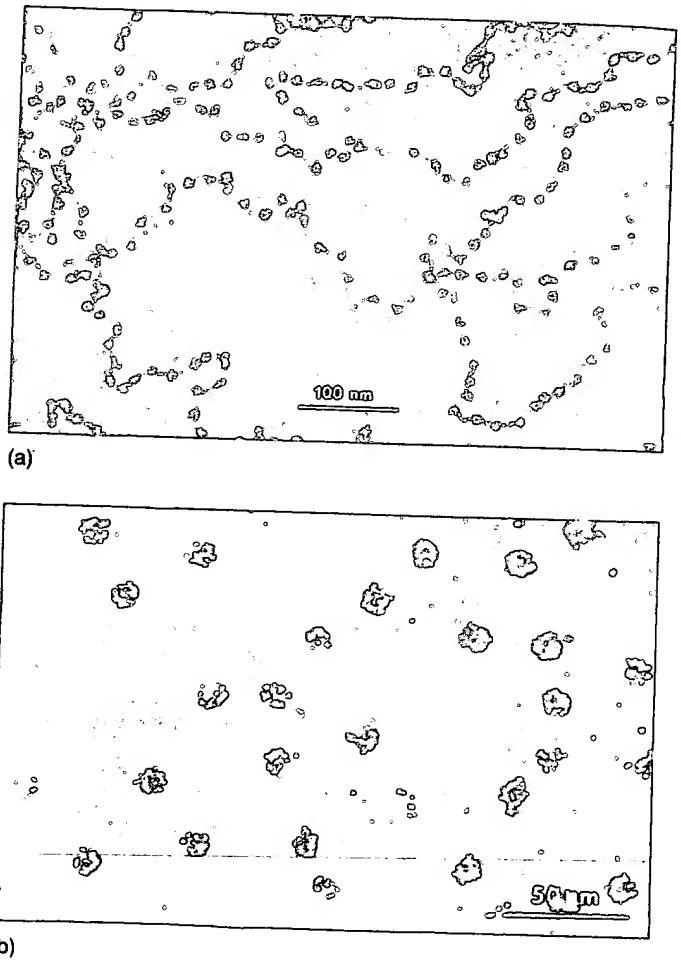


FIGURE 10.11
(a) Dark-field electron micrograph of nucleosomes present in chromatin derived from a chicken erythrocyte nucleus. (b) Dark-field electron micrograph of nucleosomes produced by micrococcal nuclease digestion. (From Olins and Olins, 1978, Figs. 1 and 4.)

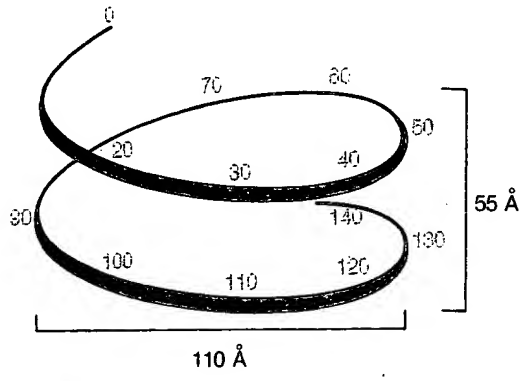


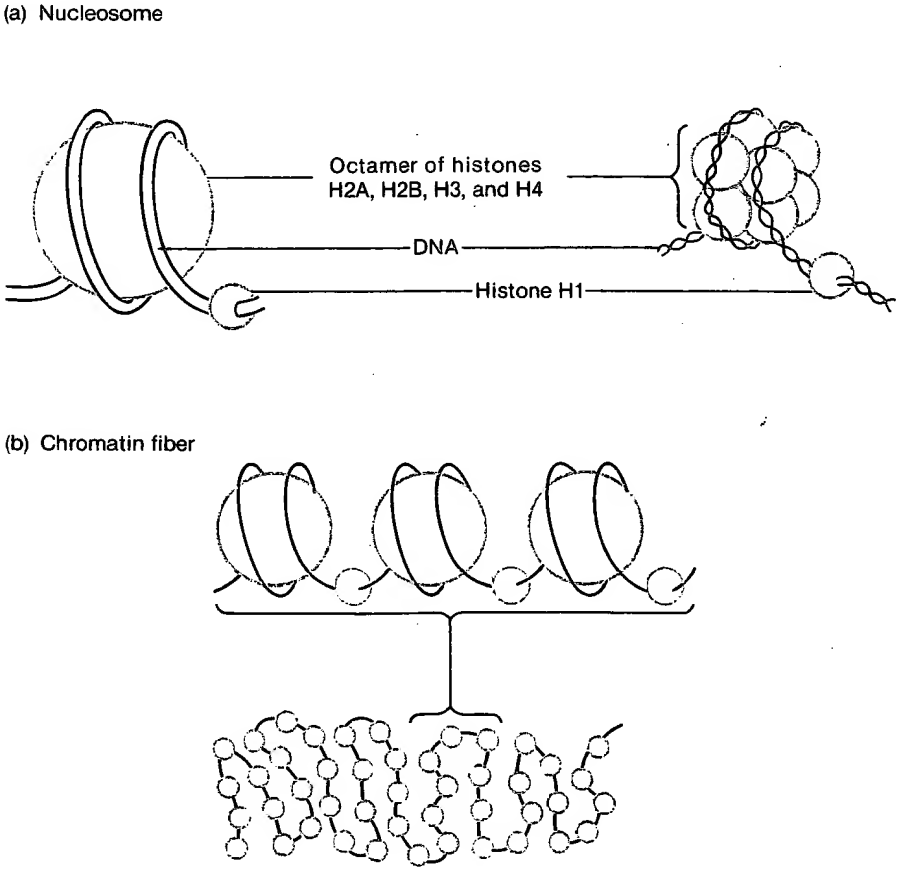
FIGURE 10.12
Representation of the DNA core of the nucleosome, called a platysome. The numbers indicate the relative positions of the 140 nucleotides contained in the core particle. (After Finch et al., 1977, Fig. 8.)

Two such micrographs appear in Figure 10.11; they show chromatin fibers composed of linear arrays of spherical particles. The particles occur regularly along the axis of a chromatin strand and are rather uniformly 70 Å in diameter. These particles, which resemble beads on a string, are now referred to as **ν-bodies** or **nucleosomes** (ν is the Greek letter *nu*). These findings conform nicely to the earlier proposal which suggested the existence of repreating units and which is supported by both the X-ray diffraction studies and the analysis of enzymatic digestion of chromatin.

3. In 1975, Roger Kornberg published the results of his study of the precise interactions of histone molecules and DNA in chromatin. His work showed that histones H2A, H2B, H3, and H4 occur as two types of tetramers: $(H2A)_2 \cdot (H2B)_2$, and $(H3)_2 \cdot (H4)_2$. He suggested that each repeating nucleosome unit consists of one of each tetramer which together interact with about 200 base pairs of DNA. These data correlate well with the two previous observations and provide the basis for a model which explains the 1:1 mass ratio of histone to DNA in chromatin.

4. Between 1975 and 1977, a more accurate understanding of the nucleosome emerged. Nuclease digestion of chromatin from diverse tissues and species was shown to yield wide variation in the number of DNA base pairs in the nucleosome unit. However, when digestion time is extended, DNA length is shortened

FIGURE 10.13
(a) General model of the association of histones and DNA in the nucleosome. (b) Schematic illustration of the way in which the chromatin fiber may be coiled into a more condensed structure.



and a core particle of about 140 base pairs is recovered from most organisms. The DNA lost in this prolonged digestion—that is, the difference in length between the nucleosome and the core particle—is now thought to link together the core particles. The histone H1 is thought to be associated with the DNA linking the core particles.

5. In 1977 J. T. Finch and other investigators, who had performed X-ray and electron microscopic analysis of crystallized core particles, proposed a model of this core particle (Figure 10.12). In this model, the 140–base-pair length of DNA is a secondary helix surrounding the core of eight histone molecules. The superhelical DNA does not quite complete two full turns, yielding a flattened wedge shape of the dimensions 57 Å × 110 Å × 110 Å. Because of this shape, the core particle of the nucleosome was called a **platysome.** Although this model does not establish the precise arrangement of histones within the helix, it provides a close approximation of the basic unit making up chromatin. The model helps us to imagine the means by which chromatin fibers can be bent, coiled, and packed in nuclei. As shown in Figure 10.13, the chromatin fiber may be converted into a uniform, supercoiled structure which may be further condensed into a mitotic chromosome.

EUKARYOTIC CHROMOSOMES: ELECTRON MICROSCOPIC OBSERVATIONS

We have so far considered what has been learned about the eukaryotic chromosome from studies involving light microscopy and from the molecular analysis of the chromatin fiber. In this section, we will examine three aspects of the eukaryotic chromosome which have been initially derived from electron microscopic observations.

THE FOLDED-FIBER MODEL FOR MITOTIC CHROMOSOMES

During the transition from interphase to mitosis, chromatin must undergo considerable compression and condensation. It has been estimated that a 10,000-fold contraction in length occurs. This process must be extremely precise given the highly ordered nature of the mitotic chromosomes in all organisms. Electron microscopic observations of mitotic chromosomes have provided an excellent overview of the intact chromosome following condensation. A whole-mount electron micrograph of a human mitotic chromosome is shown in Figure 10.14(a). In areas of greatest spreading, individual fibers similar to those seen in interphase chromatin are apparent. Very few fiber ends seem to be present. In some cases, none can be seen. Instead, individual fibers always seem to loop back into the interior. Such fibers are obviously twisted and coiled around one another, forming the regular pattern of the mitotic chromosome. The fibers are so tightly packed that, unless uncoiled slightly, the chromosome has a uniform or homogenous appearance throughout much of its length. You should compare this illustration with the light microscopic view of human chromosomes shown in Figure 2.3.

Observations of mitotic chromosomes in varying states of coiling led Ernest DuPraw to postulate the **folded-fiber model,** illustrated in Figure 10.14(c). During metaphase, each chromosome consists of two sister chromatids joined at the centromeric region. Each arm of the chromatid appears to consist of a single fiber wound up much like a skein of yarn. The fiber is composed of double-stranded DNA and protein tightly coiled together. When these intact chromosomes are viewed under the scanning electron microscope, a pattern very similar to the model emerges [Figure 10.14(b)]. An orderly coiling-twisting-condensing process appears to be involved in the transition of the interphase chromatin to the more condensed, genetically inert mitotic chromosomes. While the model may not be entirely correct, it provides an excellent illustration of what must be achieved.

SYNAPTONEMAL COMPLEX ORGANIZATION

The electron microscope has also been used to visualize an additional ultrastructural component of the chromosome seen only in cells undergoing meiosis. This structure, mentioned in Chapter 2 (Figure 2.9), is intimately associated with synapsed homologues during the pachytene stage of the first meiotic prophase and is called the **synaptonemal complex.*** In 1956, Montrose Moses observed this complex in spermatocytes of crayfish, and Don Fawcett saw it in pigeon and human spermatocytes. Because there was not yet any satisfactory explanation of the mechanism of synapsis or of crossing over and chiasmata formation, many researchers became interested in this structure. With few exceptions, the ensuing studies revealed the synaptonemal complex to be

*An alternate spelling of this term is *synaptinemal complex.*

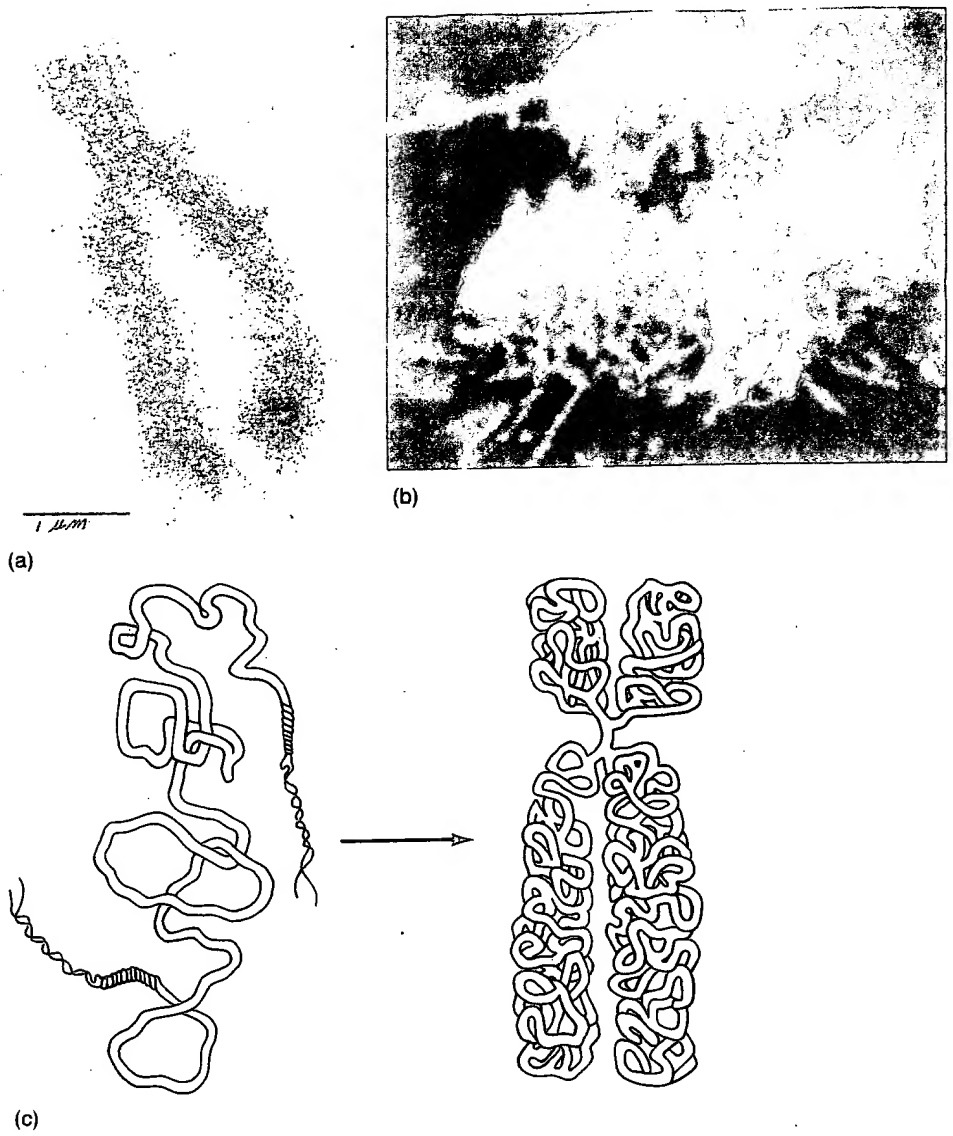


FIGURE 10.14
(a) Whole-mount transmission electron micrograph of a human mitotic D-group chromosome. (Courtesy of Walter F. Engler.) (b) Scanning electron micrograph of a human mitotic chromosome. (From Golomb and Bahr, 1971, p. 1025. Copyright 1971 by the American Association for the Advancement of Science.) (c) Depiction of the coiling and folding of the chromatin fiber into the structure characteristic of a mitotic chromosome. (Redrawn from DuPraw, 1970.)

present in most plant and animal cells visualized during meiosis.

Figure 2.9 is an electron micrograph of the synaptonemal complex. It is composed primarily of a triplet set of parallel strands. The **central element** of this tripartite structure is usually less dense and thinner (100–150 Å) than the two identical outer elements (500 Å). The outer structures, called **axial** or **lateral elements,** are distinctly and intimately associated with the chromatin of the synapsed homologues on either side. Selective staining has revealed that these axial elements contain protein and DNA. Some DNA fibrils traverse the axial elements, making connections with the central element, which is composed primarily of protein. Figure 10.15 provides a diagrammatic interpretation of the electron micrograph.

It is now generally agreed that the synaptonemal complex forms as the homologous chromosomes synapse during the first meiotic prophase stage. However, synapsis can occur in certain cases where no synaptonemal complexes are formed. Thus, it is possible that the function of this structure may be more extensive than its mere involvement in the formation of bivalents.

In certain instances where no synaptonemal complexes are formed during meiosis, synapsis is not so complete and crossing over is reduced or eliminated. For example, in *Drosophila melanogaster* meiotic crossing over rarely, if ever, occurs in males, and it is not ob-

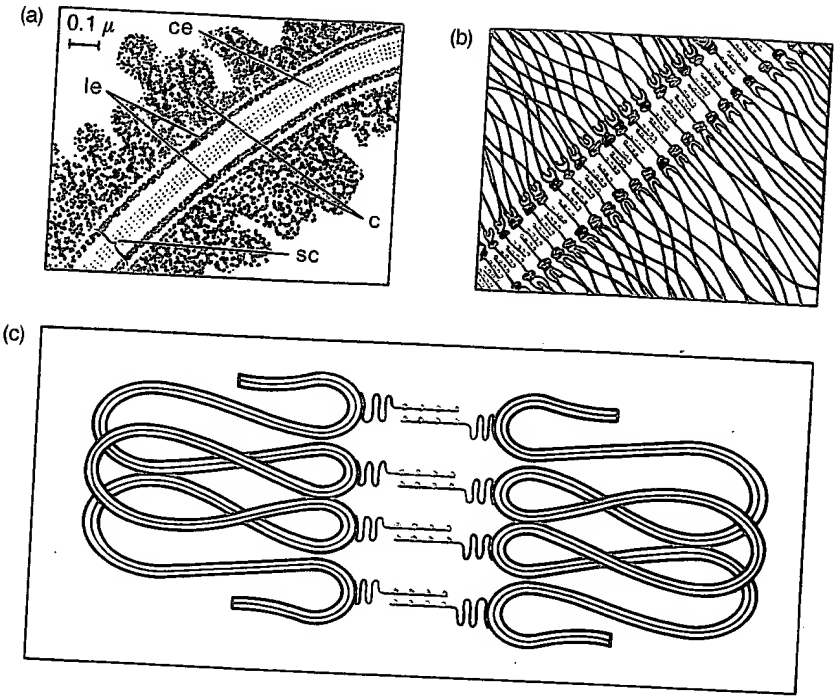


FIGURE 10.15
(a) Drawing of the synaptonemal complex (sc) observed during meiosis. Parts (b) and (c) show a schematic model of the interaction of theoretical components of this organelle with the chromatin fiber of the bivalents. [ce, central element; le, lateral element; c, chromatin] (From King, 1970, pp. 136 and 138.)

served in females homozygous for the third chromosomal mutant gene *c(3)G*. Synaptonemal complexes are not usually observed in either case. This information suggests that the synaptonemal complex must be present in order for chiasmata to form and crossing over to occur. In addition, it has been hypothesized that the genetic product coded for at the *c(3)G* locus may in some way be involved in the biosynthesis of the complex.

There may be other, yet undiscovered molecular components that are essential to the function of the synaptonemal complex. Even so, the discovery of the complex is significant to the field of genetics. If the complex is critical to synapsis during meiosis, then all sexual reproduction is dependent on it. If the complex is essential to crossing over, then it is largely responsible for eukaryotic genetic recombination, enhancing the role of that process in evolution.

NA OF MITOCHONDRIA ND CHLOROPLASTS

e results of a number of experiments have demonted that both **mitochondria** and **chloroplasts** contain r own genetic information. It was observed that cermutations in yeast, other fungi, and plants corred with altered functioning of mitochondria or the roplasts. These mutations were not always inherin the ratios expected by Mendelian principles. Ofthese traits seemed to follow a **maternal mode of ritance**; that is, the mutation would be passed on if it were possessed by the female parent. When it became clear that both mitochondria and chloroplasts are self-replicating, it seemed logical that these organelles might contain their own genetic information.

Later autoradiographic studies showed that chloroplasts and mitochondria could incorporate radioactive DNA precursors. Thus, geneticists set out to look for DNA in these organelles with the electron microscope. Electron microscopists not only documented the presence of DNA in both organelles, but they also saw DNA in a form quite unlike that seen in the chromosomes of eukaryotic cells.

Mitochondria are smaller than chloroplasts and contain less DNA. **Mitochondrial DNA (mtDNA)** has a form almost identical to the DNA seen in some viruses. It is a circular fiber with a diameter roughly equivalent to that predicted for a double helix. Figure 10.16(a) shows a molecule of mtDNA. The mitochondrial DNA molecule is about 5 μm long and contains enough DNA to code for about 15 to 20 gene products. As we will learn in Chapter 15, many of these products, including respiratory enzymes, ribosomal proteins, transfer RNAs, and ribosomal RNA, have been identified.

DNA released from chloroplasts is much longer than mitochondrial DNA, as shown in Figure 10.16(b). Because of its size, chloroplast DNA has been more difficult to characterize. Like mtDNA, chloroplast DNA is circular in most, if not all, plants.

Because mitochondrial and chloroplast DNA are similar in appearance to that of bacteria, a theory of the origin of these organelles has been proposed. This hypothesis, called the **endosymbiont theory**, states that

mitochondria and chloroplasts may have originated as bacterial-like particles which became incorporated into eukaryotic cells. In the evolution of this symbiotic relationship, the particles lost their ability to function independently. We will discuss the genetics of mitochondria and chloroplasts in more detail in Chapter 15.

HETEROCHROMATIN

All recent evidence supports the concept that the DNA of each eukaryotic chromosome consists of one continuous double-helical fiber along its entire length. A continuous fiber is the basis of the **unineme model** of DNA within a chromosome and is in keeping with what we have seen in viral and bacterial chromosomes. This finding might lead one to suspect that the chromatin fiber of each unit would demonstrate structural uniformity along its length. However, in the early part of this century, it was observed that some parts of the fiber remain condensed and stain deeply during interphase, but most do not. In 1928, E. Heitz coined the terms **heterochromatin** and **euchromatin** to describe the parts of chromosomes which remain condensed and those which are uncoiled, respectively.

Subsequent investigation has revealed a number of characteristics of heterochromatin which distinguish it from euchromatin. Heterochromatin areas are genetically inactive either because they lack genes or contain genes that are repressed. Also, heterochromatin replicates later during the *S* phase of the cell cycle than does euchromatin. This characteristic has been established using autoradiography and is thought to be a secondary feature of the extreme condensation. The discovery of heterochromatin provided the first clues that parts of eukaryotic chromosomes may not be related to storage of information. Instead, it is believed that some chromosome regions may be involved in the maintenance of the chromosome's structural integrity.

Heterochromatin is found in the genetic material of every species. Early cytological studies showed that areas closely adjacent to the centromeres were composed of heterochromatin. The ends of chromosomes, called **telomeres,** are also considered to be heterochromatic. In certain cells, notably the polytene-chromosome-containing salivary gland cells of *Drosophila,* the heterochromatin of centromeres may clump together to form a **chromocenter** (see Figure 10.5). This observation suggests that some molecular affinity involved in heterochromatin is responsible for this adherence.

Heterochromatin has been subdivided into two types. **Constitutive heterochromatin** is present at homologous sites on pairs of chromosomes and always exists in a genetically inert state. This form has been shown to contain DNA sequences which are not organized into genes. Telomeres and centromeric regions are examples of constitutive heterochromatin.

The second type of heterochromatin, called **facultative,** is chromatin with the faculty, or potential, to become heterochromatic. For example, one of the two X

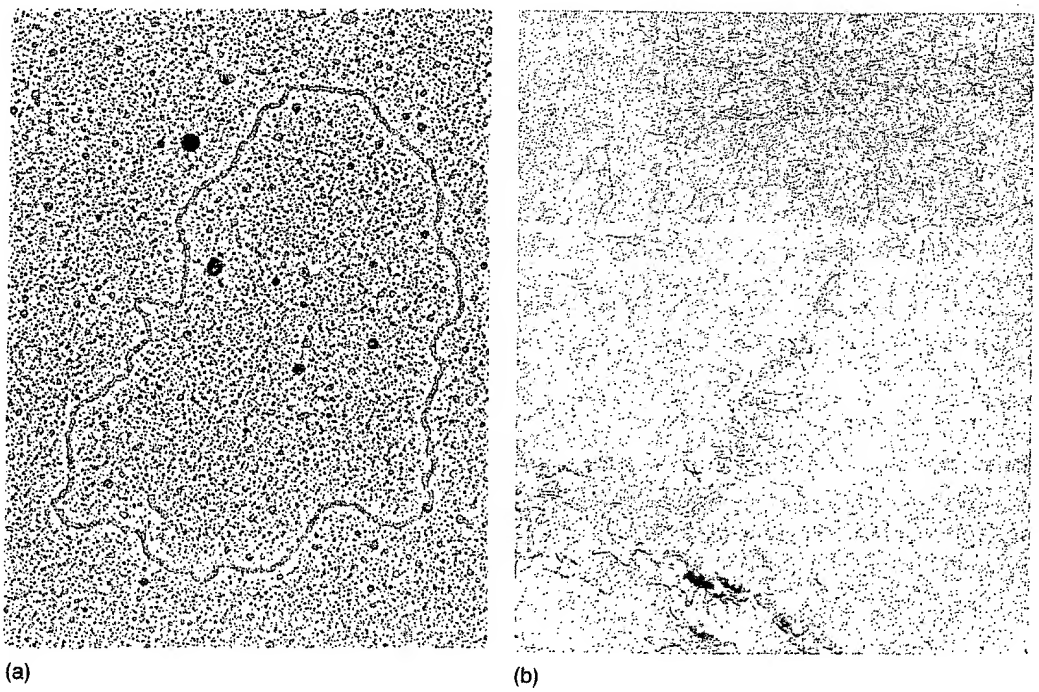


FIGURE 10.16
(a) Electron micrograph of mitochondrial DNA (mtDNA). [From Nass, 1970, Plate Ia. Copyright: Academic Press Inc. (London) Ltd.] (b) Electron micrograph of chloroplast DNA derived from *Acetabularia.* (Courtesy of Beverly R. Green.)

chromosomes in mammalian females exhibits this property when a **Barr body** is formed. In certain species, such as the mealy bug, one entire haploid set of chromosomes becomes heterochromatic. In both of these examples, only one member of a homologous pair or set of chromosomes becomes heterochromatic, in contrast to constitutive heterochromatin. The Y chromosome of many speices, lacking a true homologue, is classified as facultative heterochromatin. It remains condensed during interphase, and the majority of its length is genetically inert.

Facultative heterochromatin may contain genetic information, but it is not used by the organism once it becomes condensed. It is believed that the extreme condensation and coiling of heterochromatic material physically precludes genetic activity in the same way that the condensation of mitotic and meiotic chromosomes renders them inactive.

When certain heterochromatic areas from one chromosome are translocated to a new site on the same or another nonhomologous chromosome, genetically active areas sometimes become genetically inert if they now lie adjacent to the translocated heterochromatin. This influence on existing euchromatin is one example of what is more generally referred to as a **position effect.** That is, the position of a gene or groups of genes relative to all other genetic material may affect their expression. This topic is discussed more extensively in Chapter 12.

HETEROCHROMATIN, SATELLITE DNA, AND REPETITIVE DNA

Chapter 8 and Appendix A describe techniques useful in the analysis of DNA. The use of two of these—sedimentation equilibrium centrifugation and reassociation kinetics—has provided important information about the nature of DNA in chromatin. Specifically, the DNA of heterochromatin and euchromatin differs in molecular composition. With other techniques, functional differences between the two forms of chromatin have been determined. The nucleotide composition of the DNA of a particular species is reflected in its density, which can be measured with sedimentation equilibrium centrifugation. When the DNA of any eukaryotic species is analyzed in this way, the majority of it shows up as a single main peak or band of uniform density. However, one or more satellite peaks represent DNA that differs slightly in nucleotide composition and density from main-band DNA. This minor component, called **satellite DNA,** seldom represents more than 10 percent of the total DNA. A profile of main-band and satellite DNA from the mouse is shown in Figure 10.17.

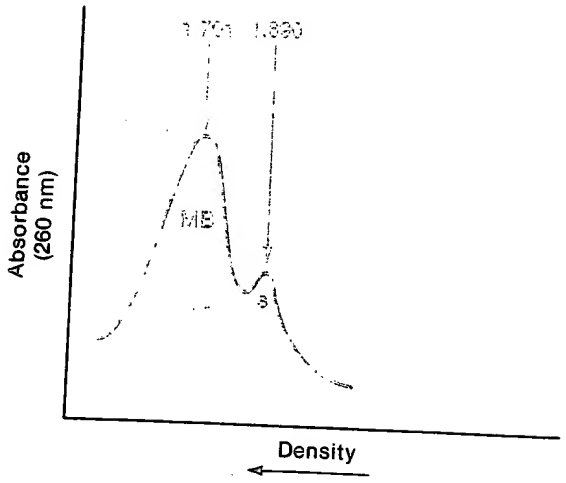


FIGURE 10.17
Separation of main-band (MB) and satellite (s) DNA from the mouse using sedimentation equilibrium centrifugation of a CsCl gradient.

The significance of satellite DNA remained an enigma until the mid 1960s, when the techniques for measuring the reassociation kinetics of DNA became available. These techniques, developed by Roy Britten and David Kohne, allowed geneticists to determine the rate of reassociation of fragmented DNA. Britten and Kohne showed that when complementary strands of DNA fragments of uniform length were separated by heating and then reassociated by cooling, certain portions were capable of reannealing more rapidly than others. They concluded that rapid reannealing was characteristic of multiple DNA fragments composed of identical or nearly identical nucleotide sequences—the basis of **repetitive DNA.**

The proportion of the genome occupied by repetitive sequences varies widely in eukaryotes. Most of the DNA in lower eukaryotes consists of unique sequences present only once in the genome. In most animals, however, up to 50 percent of all DNA is present in repetitive form. In some plants and amphibians, this figure is even higher.

While there are even finer distinctions made, repetitive DNA is often classified into two major categories. **Highly repetitive DNA** is present between 100,000 and 1,000,000 times in the genome and consists of short sequences 5 to 300 nucleotide pairs (np) in length. Most frequently, the sequences are between 5 and 15 np long. Highly repetitive DNA is considered to consist of untranscribed sequences and therefore does not consist of genes. Such repeated sequences are usually found clustered in distinct regions of chromosomes.

The second category is called **moderately or middle repetitive DNA.** It is present 10 to 3000 times in the genome and most frequently between 10 to 100 times. On the average, these sequences consist of about 300 np. At least some of the moderately repetitive sequences are transcribed and thus constitute repeat or duplicate copies of genes.

Nonrepetitive DNA, usually containing 1000 or more nucleotide pairs, is said to consist of **unique sequences.** They are present only once, or a very few times, in the genome and are believed to represent structural genes coding for a variety of RNA and protein products.

This information raises two intriguing questions: (1) Can satellite DNA be correlated with repetitive DNA? and (2) If so, can both be correlated with the phenomenon of heterochromatin? The answer to both questions is yes. When satellite DNA is subjected to analysis by reassociation kinetics, it falls into the category of highly repetitive DNA. It therefore consists of short sequences repeated a large number of times. The available evidence further suggests that these sequences are present as tandem repeats clustered at various positions in the genome.

Satellite DNA is found in very specific chromosome areas known to be heterochromatic—the **centromeric regions.** This was discovered in 1969 when several workers, including Mary Lou Pardue and Joe Gall, applied the technique of ***in situ* hybridization** to the study of satellite DNA. This technique (see Appendix A) involves the molecular hybridization between an isolated fraction of radioactive DNA or RNA and the DNA contained in the chromosomes of a cytological preparation. Following the hybridization procedure, autoradiography is performed to locate the chromosomal areas complementary to the fraction of DNA or RNA.

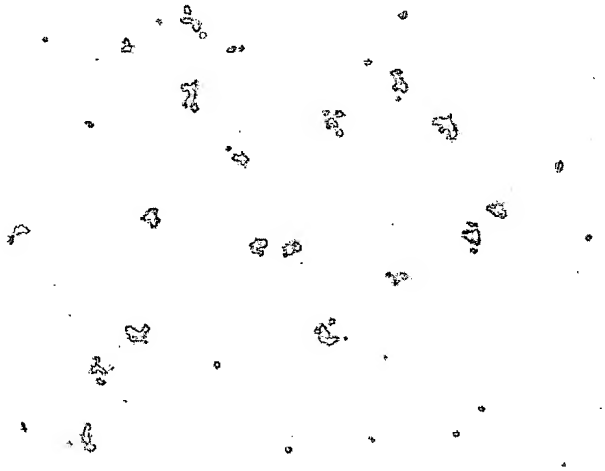


FIGURE 10.18
In situ hybridization between RNA transcribed by mouse satellite DNA and mitotic chromosomes. The grains in the autoradiograph localize the chromosome regions containing satellite DNA sequences. (From Pardue and Gall, 1972. Copyright Keter Publishing House Jerusalem Ltd.)

In their work, Pardue and Gall demonstrated that RNA transcribed by mouse satellite DNA hybridizes with DNA of centromeric regions of mouse mitotic chromosomes (Figure 10.18). Thus, several conclusions can be drawn. Satellite DNA differs from main-band DNA in its molecular composition, as established by buoyant density studies. It is also composed of short repetitive sequences. Finally, satellite DNA is found in the heterochromatic centromeric regions of chromosomes.

These conclusions establish the basis of the structural and functional differences between heterochromatin and euchromatin. They further reflect the complexity of the genetic material in eukaryotes. Instead of viewing the chromosome as a linear series of genes, we must now see it as quite variable in its molecular organization and functional capacity. For example, there are nucleotide sequences that do not specify gene products. Are these genetically inert DNA sequences of heterochromatin related to the maintenance of the structural integrity of chromatin? Or, might they be involved in a regulatory role of transcription? Such noninformational sequences may play more than one role. There is no doubt that more extensive investigation will continually expand and modify our concept of eukaryotic genetic organization.

HETEROCHROMATIN IDENTIFICATION: CHROMOSOME BANDING

Until about 1970, mitotic chromosomes viewed under the light microscope could be distinguished only by their relative sizes and the positions of their centromeres. In organisms with a low haploid number, two or more chromosomes are often indistinguishable from one another. However, around 1970, differential staining along the longitudinal axis of mitotic chromosomes was made possible by new cytological techniques. These methods are now called **chromosome banding techniques** because the staining patterns are similar to the bands of polytene chromosomes.

One of the first chromosome banding techniques was discovered by Pardue and Gall during the devel-

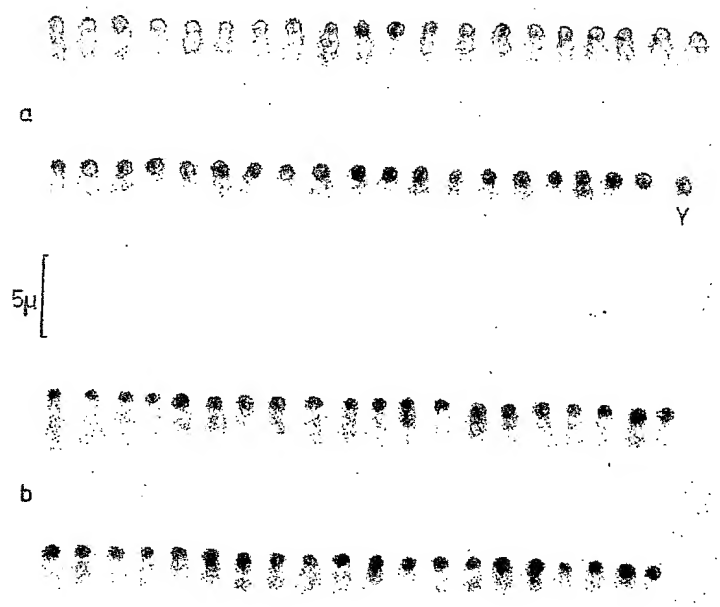


FIGURE 10.19
Karyotypes of a male (a) and female (b) mouse where chromosome preparations were processed and stained to demonstrate C bands. (From Chen and Ruddle, 1971, p. 54.)

opment of the *in situ* hybridization procedure. They discovered that if chromosome preparations were heat denatured and then treated with Giemsa stain, a unique staining pattern was observed. The centromeric regions of mitotic chromosomes preferentially took up the stain! Thus, this cytological technique stained a specific area of the chromosome composed of constitutive heterochromatin. A diagram of the mouse karyotype treated in this way is shown in Figure 10.19. The staining pattern is referred to as **C-banding.**

Still other chromosome banding techniques were developed about the same time. A group of Swedish researchers, led by Tobjorn Caspersson, used a technique which provided even greater staining differentiation of metaphase chromosomes. They used fluorescent dyes which bind to nucleoprotein complexes and produce unique banding patterns. When the chromosomes are treated with the flurochrome **quinacrine mustard** and viewed under a fluorescent microscope, precise patterns of differential brightness are seen. Each of the 23 human chromosome pairs can be distinguished by this technique. The bands produced by this method are called **Q-bands.**

Another banding technique, which produces a staining pattern nearly identical to the Q-bands, has been developed by Tau-Chiuh Hsu and Frances E. Arrighi. This method, producing **G-bands** (Figures 10.20 and 10.21), involves the digestion of the mitotic chromosomes in the cytological preparations with the proteolytic enzyme **trypsin** followed by Giemsa staining. Another technique results in the reverse G-band staining pattern, called an **R-band** pattern. In 1971, a meeting was held in Paris to establish the nomenclature for these various patterns. Still other banding techniques are now available.

Intense efforts are currently underway to elucidate the molecular mechanisms involved in producing these banding patterns. The variety of staining reactions under different conditions reflects the heterogeneity and complexity of chromosome composition. The variations in nucleic acid composition that exist along the longitudinal axis of any given chromosome, with its alternating sections of heterochromatin and euchromatin, suggest functional diversity. As additional information becomes available, a clearer and more unified concept of genetic organization is expected.

DIRECTION OF FUTURE STUDIES

Recent investigations have transformed the study of euchromatin and heterochromatin from an interesting but poorly understood topic into one of the most exciting areas of genetics. The banding techniques which distinguish regions on metaphase chromosomes allow cytogeneticists to detect chromosomal aberrations with greater precision. This knowledge can also be applied to the study of evolution. It is now possible to observe differences in certain chromosome regions from organisms at different points in the phylogenetic scale and to determine precisely similarities of karyotypes between closely related species.

In Chapter 19, we will probe further into the structure of "classical" genes, which store transcribable genetic information. These genes constitute the euchromatic segments of chromosomes which contain unique nucleotide sequences.

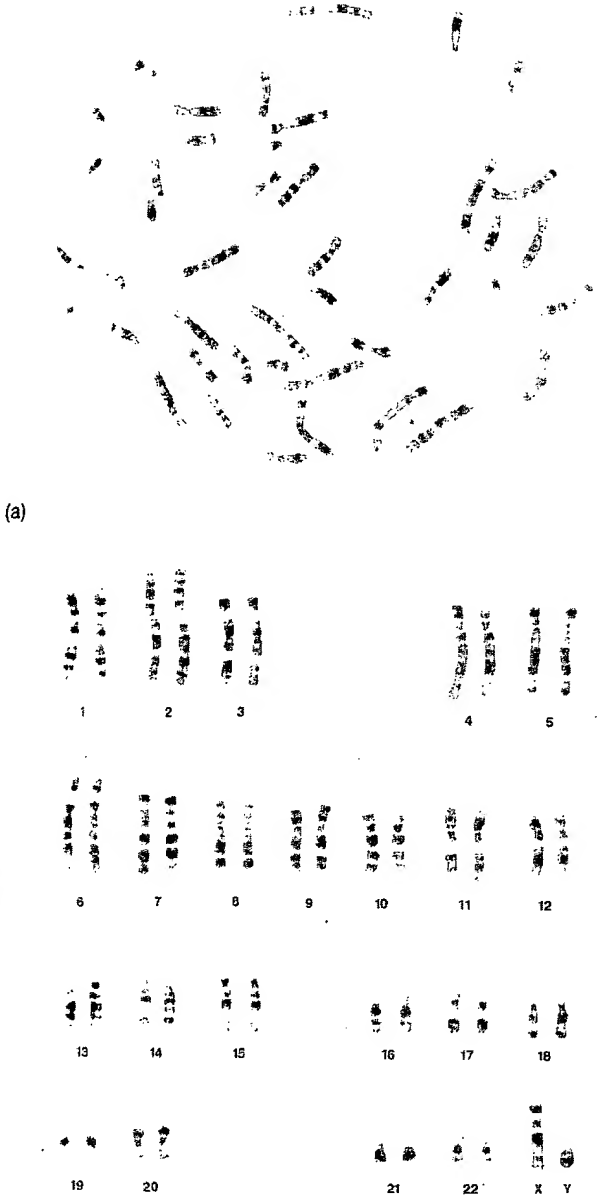


FIGURE 10.20
(a) G-banded human metaphase preparation. (b) G-banded karyotype of a normal male showing approximately 400 bands per haploid set of chromosomes. Chromosomes were derived from cells in midmetaphase. (From Yunis, 1981.)

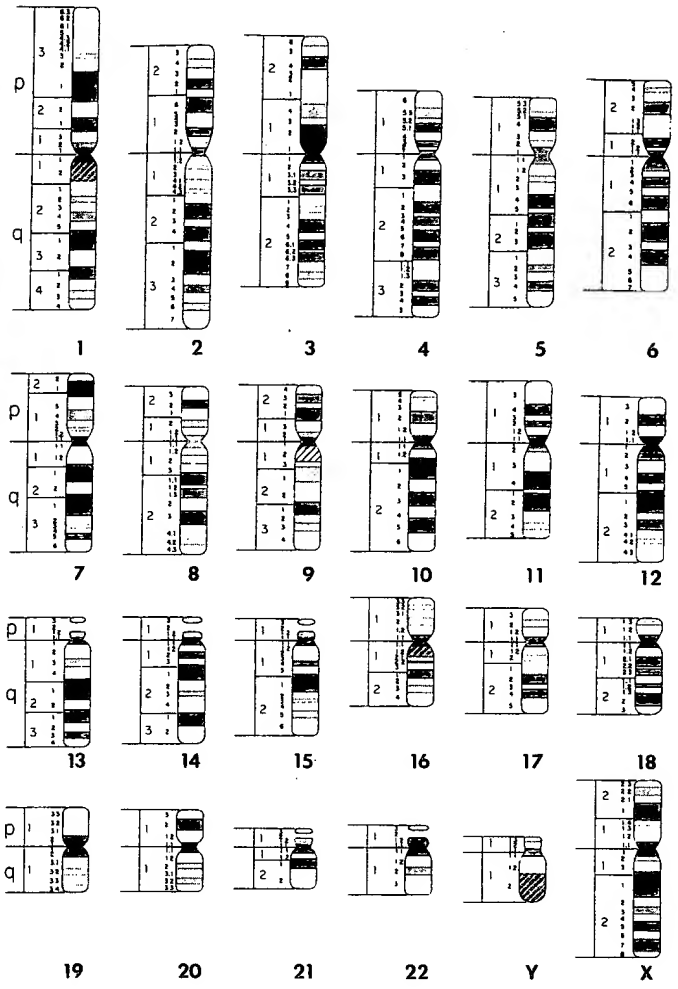


FIGURE 10.21
Schematic representation and nomenclature of human chromosomes at the 400-band stage using G-banding. Different shades represent varying intensities of bands. (From Yunis, 1981.)

SUMMARY AND CONCLUSIONS

The organization of the molecular components of chromosomes has been of great interest from the time of their discovery. Knowing how these components are organized will help us to understand how the genetic material functions. Visualization of viral and bacterial chromosomes under the electron microscope has revealed rather simple, usually circular DNA helices which correspond closely to the Watson-Crick model of this molecule. The more classical studies of the gross structure of mitotic figures, polytene chromosomes, and lampbrush chromosomes relied on light microscopic observations. These studies have provided valuable insights into the morphology of eukaryotic chromosomes. Mitotic figures may be arranged into the karyotype of any organism. The study of karyotypes has provided important clues concerning the genetic basis of certain human disorders and has been important to evolutionary studies. The appearance of bands and puffs in polytene chromosomes and loops in lampbrush chromosomes has been correlated with gene activity. Recently,

Z-DNA has been found to occur in interband regions of polytene chromosomes.

Biochemical analysis of the eukaryotic chromatin fiber has revealed that it is a nucleoprotein. Histone and nonhistone proteins are intimately bound to DNA. Histones, positively charged proteins, are part of a repeating unit along the chromosome axis, the nucleosome. As such, histones are important to the structural integrity of the fiber. The nucleosome may be important in facilitating the conversion of the extensive chromatin fibers characteristic of interphase into the highly condensed chromosomes seen in mitosis. The folded-fiber model, based on electron microscopic observations, suggests how such a transition takes place. Other ultrastructural observations have revealed the presence of the synaptonemal complex during meiosis in all organisms where synapsis and crossing over occur. Through the use of electron microscopy, DNA has been found in mitochondria and chloroplasts. It is similar in appearance to that of viruses and bacteria.

Biochemical and cytological investigations have extended our knowledge of functional and structural heterogeneity along the axis of chromosomes. Genetically inert heterochromatin has been clearly distinguished from potentially active euchromatin, and new insights into the structure of heterochromatin have been gained. In some instances, heterochromatin is composed of repeating or repetitive nucleotide sequences and can be identified cytologically. Chromosome banding techniques have confirmed that the axis of the mitotic chromosome is not chemically homogeneous. These findings are also significant to other areas of genetics.

Problems and Discussion Questions

1 Compare and contrast the chemical nature, size, and form assumed by the genetic material of viruses and bacteria.

2 Why might it be predicted that the organization of eukaryotes' genetic material would be more complex than that of viruses or bacteria?

3 Describe the formation of polytene chromosomes. What do the terms *band, interband, chromomere,* and *puff* signify?

4 Correlate the observed structure of the lampbrush chromosome complex with what you have learned about the first meiotic prophase in Chapter 2. How do the enzyme digestion experiments support this model?

5 Describe the sequence of research findings leading to the most recent model of chromatin structure. What is the molecular composition and arrangement of the nucleosome?

6 Speculate as to why a structure such as the synaptonemal complex is necessary for precisely accomplishing synapsis and crossing over.

7 When chloroplasts and mitochondria are isolated, they are found to contain ribosomes which are similar to prokaryotic cells. What does this suggest about the function of DNA contained in these organelles? Does this observation support the endosymbiont theory?

8 Provide a comprehensive definition of heterochromatin.

9 List examples of constitutive and facultative heterochromatin.

10 Why are the cytological banding techniques considered to be very significant to the study of genetics?

Selected Readings

ARRIGHI, F. E., and HSU, T. C. 1971. Localization of heterochromatin in human chromosomes. *Cytogenetics* 10: 81–86.

BEERMAN, W., and CLEVER, U. 1964. Chromosome puffs. *Scient. Amer.* 210: 50–58.